Quantification of Dermal Absorption of Pesticides from Dried Residues

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Abstract

Pesticides must go through a rigorous risk assessment process to demonstrate that they are safe for use. One exposure scenario is that of the re-entry worker, who may enter a field soon after pesticide application and come in to contact with contaminated foliage. Following estimation of the potential dermal exposure, prediction of a systemic dose relies on applying a factor for dermal absorption. Currently, this value is obtained by carrying out *in vitro* diffusion cell testing of the concentrate and one or more representative spray dilutions. However, a worker is exposed to pesticide in a different form to those tested, a dried residue. The current work has developed an *in vitro* protocol to measure the absorption of pesticides from dried residues. This method is based on applying pesticides to an inert platform, creating dried deposits of pesticide, which are then transferred to a skin membrane and absorption measured *in vitro* using Franz diffusion cells.

This method has been used to compare the dermal absorption of four compounds, from spray dilutions and their residues. In each case absorption was significantly less from the residue than the spray dilution. Further investigation of two of these compounds, found that absorption was affected by formulation and loading dose. Additionally, the effect of decontaminating the skin at different time points post-exposure found an effect on the total amount absorbed and that this effect may be more pronounced for the dried residue than the spray dilution. This work has provided valuable insight into an area of exposure science which is poorly documented. This novel method has the potential to be used to carry out more realistic risk assessments than those which may currently overestimate exposure of re-entry workers and hinder the passage of safe and effective products through the registration process.

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List of Abbreviations and Acronyms

AI - Active Ingredient

AFM – Atomic Force Microscopy

AOEL - Acceptable Operator Exposure Level

CFZ – Clodinafop propargyl

D - Diffusivity

Da – Daltons

DDT – dichlorodiphenyltrichloroethane

DFR – Dislodgeable Foliar Residue

DFZ - Difenoconazole

EC-A – Emulsifiable Concentrate A

EC-B – Emulsifiable Concentrate B

EFSA – European Food Safety Authority

EU - European Union

ha - Hectare

Log P - log octanol-water partition coefficient

LOQ – Limit of Quantitation

MW – Molecular Weight

NR – Nile Red

OECD - Organisation for Economic Co-operation and Development

PBS – Phosphate buffered saline

PDE – Potential Dermal Exposure

PET – Polyethylene Terephthalate

PG – Polyethylene glycol

PPZ - Propiconazole

SEM – Scanning Electron Microscope

SC – Stratum Corneum

SRS – Stimulated Raman Scattering

T – Time

TC – Transfer Coefficient

TXP – Trinexapac-ethyl

WP – Wettable Powder

1 BACKGROUND

1.1 Pesticides

Humans are arguably the most successful organism on the planet, success which we owe to our ability to manipulate our surrounding environment to suit our needs. As the human population has grown, the need to increase our supply of food has necessitated changes in the way we grow and harvest crops. The introduction of monoculture, the growth of one single crop type in a given area, represents a large increase in crop output. However, vast changes to the environment like this can disrupt the natural equilibrium and nature will react accordingly.

In a 'natural' ecosystem each organism fills its own niche within the environment, and grows in population size to fill that niche. The limiting factor for growth would usually be space, light, supply of nutrients and water, or predation. Each ecosystem will reach an equilibrium where populations of each organism remain relatively constant. As with any equilibrium, if an external force is applied it will change accordingly.

When a wild area is transformed into a monoculture, many organisms will lose their habitat. Any organism present in this area that is not the desired output crop; animals that had nested or plants and fungi that had grown in this area, are removed. If subsequently left untouched, plants, fungi and animals that originally grew there would eventually recolonize the area, unless the introduced crop happens to be better adapted to that area, which is rarely the case. However, in the short term, the planting of a large quantity of a single crop has changed this environment. Due to this change, animals that can feed on the introduced crop or other organisms that can use it to their advantage could see a rapid

increase in population. This effect is exacerbated in some instances where the natural predators of these organisms are forced out of the area. These few organisms that are able to flourish in this new environment are now referred to as 'pests'. A system will return to equilibrium in the absence of an external driving force. So, in order to maintain a 'pest-free' environment and increase the yield of the desired crop, humans have long been applying this external driving force in the form of pesticides.

1.1.1 History & Uses

A pesticide is a chemical that is deliberately applied to an environment to elicit an effect, most often death, upon a pest. The most common motivation for the use of pesticides is to increase crop yields and the shelf-life of foods. They are also used for aesthetic reasons, for example to maintain the grass of a golf course or remove weeds on a driveway. Pesticides may also be used to control populations of disease-causing organisms such as mosquitos or head lice. The latter use is particularly interesting in that the pesticides can be directly applied to the skin of a human in order to elicit their effects.

As early as 4000 years ago, humans were reported to use sulphur dusting as means of controlling insects[2]. Throughout history examples of other pesticides can be found, a medical document from ancient Egypt, 'The Ebers' papyrus' details many remedies that were used to control and kill pests[3].

Arsenic-based compounds were used as insecticides as early as A.D 900 and into the 20th century. Paris Green or Emerald green (Figure 1) was commonly used as an insecticide through the 19th century. It was also commonly used as a dye at this time, proving popular with artists including Monet and van Gogh, and was commonly used in the manufacture of green textiles and wallpapers with little knowledge of its toxicity. There are anecdotal reports of deaths caused from its use in culinary applications such as on cake decorations[4].

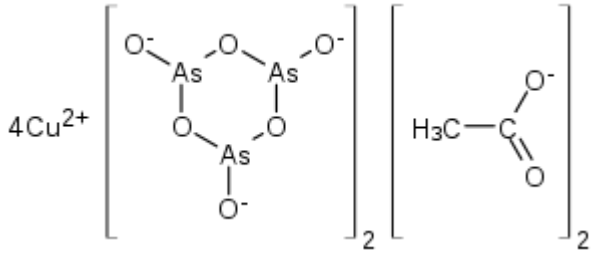

Figure 1 - Copper (II) acetoarsenite (Paris Green) chemical structure

In the 1880s, Paris Green was largely replaced by lead arsenate, which was often made at home by combining lead salts and sodium arsenate[5]. With the introduction of sprayers at the turn of the century, demand increased for commercially formulated pesticides that sprayed well. Most of the compounds used around this time such as mercury and arsenic compounds are no longer used due to their unacceptable toxicity. One pesticide that has stood the test of time is 'Bordeaux mixture', containing copper sulphate and slaked lime which is still used today (Figure 2).

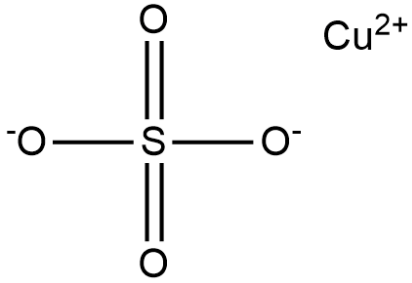

Figure 2 - Copper (II) sulphate (Bordeaux Mixture) chemical structure

In 1939, Paul Müller discovered the pesticidal properties of dichlorodiphenyltrichloroethane (DDT) for which he was awarded a Nobel Prize in 1948 (Figure 3). DDT, a broad spectrum insecticide was used during World War II to curb the spread of malaria and was used widely, some may say over-used, in agriculture for many years.

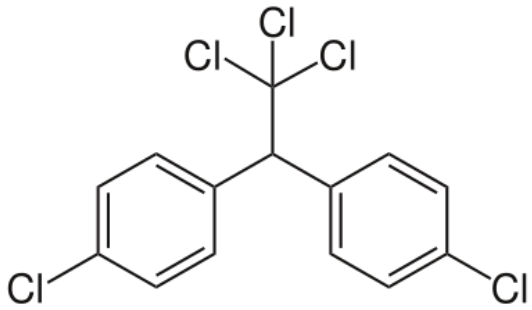

Figure 3 - dichlorodiphenyltrichloroethane (DDT) chemical structure

In the post-war period, several other compounds including parathion, atrazine, paraquat and 2,4-D emerged. These compounds were often applied liberally to large areas in attempts to control many different types of pest. Although relatively effective, this pattern of use was unsustainable, resulting in some organisms developing resistance and, due to the 'broad spectrum' of many of these compounds, their off-target effects were far reaching. Many non-pest organisms were inadvertently affected and ecosystems damaged, a topic which was bought to the fore in 1962 by Rachel Carson's "The Silent Spring"[6] which is generally acknowledged as signalling the beginning of modern pesticide regulation[2].

Due to increased regulation in recent times, there has been a move toward developing compounds that are more specific to their target and persist in the environment for less time. This means that the potential for off-target toxicity is much lower. Also, advances in spraying and formulation technology mean that it is possible to gain the same efficacy by applying significantly less product.

Toxicity caused by exposure to pesticides varies by compound and species. Adverse effects in humans can be seen acutely upon exposure to high doses, such as nausea and vomiting. Chronic exposure can cause long term adverse effects such as neurotoxicity or liver damage. These toxicities are often caused by the compound affecting the same pathways that makes them so effective as pesticides. In the case of an insecticide for example, dose is key: a dose that would kill an insect may not cause any tangible acute toxicity in a human. However, it

is difficult to study the effects of long term low level exposure to these compounds and exposure must be reduced wherever possible.

1.1.2 Risk Assessment

Each pesticide product must go through a rigorous risk assessment process to be deemed safe for its intended use. There are many models and assessment guidelines available, and each country or governing body has different approaches for exposure assessment. For the purpose of this thesis, current practise for the European Union (EU) will be discussed only. Exposure assessment in the EU is overseen by the European Food Safety Authority (EFSA) and in 2014 a new guidance document on pesticide exposure assessment was released[7]. Guidance on dermal absorption was released in 2012[8] based on a scientific opinion document released the year before[9]. This guidance was updated in 2017[10] in consideration of a large dataset held by Industry and the German regulatory authority, BfR (Bundesinstitut für Risikobewertung). Much of the information in this section is based on these documents.

1.1.2.1 Modes of Exposure

Exposure to pesticides can occur in many different scenarios and to several different groups of individuals. Oral exposure is the route which is most within the public consciousness; many fruits and vegetables may contain small amounts of pesticide, which could potentially be consumed by the public. Dietary risk assessment is discussed in detail in further EFSA documents[11, 12].

Once a pesticide has been licensed for sale, there are many groups of individuals who may inadvertently become exposed to the product. First, workers in the manufacturing plants where the compound is produced and formulated may be at risk of exposure as part of their daily work. Depending on the type of pesticide and its intended use there is a vast array of possible exposure scenarios for the

end-use product including use by farmers, pest control professionals and the public. In this document, the use of pesticides to be sprayed onto crops in a commercial environment will be discussed. Most products are supplied in the form of a concentrate which must first be diluted with water before application. This task would be carried out by an 'operator', who loads the concentrate into the spray tank and mixes it to a suitable spray dilution, usually with water as the diluent. This spray tank could be in the form of a handheld sprayer or knapsack, or a large tractor mounted tank. During this process the operator is typically exposed to the concentrate, although exposure to the spray dilution could also occur with less sophisticated equipment. However, during the application process which ensues, exposure is primarily to the diluted spray solution. Equally, post-application there is a risk of exposure when emptying and cleaning the spray reservoir.

Inhalation exposure can occur during the mixing and loading process, if the active ingredient is volatile in nature or a fine powder. However, the most likely time for inhalation exposure is during the spraying process. Upon spraying, very fine droplets can become airborne and subsequently inhaled. It is also possible for bystanders and residents to be exposed via inhalation if significant spray drift occurs. Due to the high surface area of the lungs and its relatively minimal barrier function, these exposures could potentially cause significant toxicity. In recent times, inhalation exposure has been reduced due to the introduction of closed cabs, which reduce exposure of the operator, and more efficient spray equipment which reduces spray drift.

Although the oral and inhalation routes of exposure are both important pathways, they are not considered further here. Only dermal exposure, which is the principal route for most non-dietary exposure scenarios, is discussed in this thesis.

1.1.3 Dermal Exposure Assessment

Dermal exposure can occur from spillage of a product directly onto the skin or by dermal contact with a surface that has been contaminated. During the mixing and loading process it is possible for a worker to be dermally exposed to either the concentrate or its spray dilution, i.e., when diluted in water for spraying, this would usually occur via splash or spill. As described above for inhalation, operators, bystanders or residents could also be dermally exposed via spray drift. Again, this has become less common in recent times and precautions can be taken such as appropriate engineering controls, the use of drift reduction technology, increasing droplet size or lowering the boom height to reduce spray drift.

This thesis will focus specifically on dermal exposure of re-entry workers. Post-application, it is necessary for these workers to enter the treated area to carry out tasks such as crop inspection or harvest. It is also possible that bystanders could be exposed; for example, hikers passing through a field who may be unaware that it is has been sprayed recently. This type of exposure is different to those described above. Upon contact with foliage, a worker is not exposed to either the concentrate or the spray dilution, but rather to a 'dried residue' of the spray dilution. 'Dried residue' here is defined as a form of the spray dilution where all the volatile components of the formulation have evaporated away. At the least, this means that the water from the dilution has evaporated but, for some formulations, other components may have also evaporated. It is therefore apparent that exposure to this dried residue may be distinctly different to exposure to the spray dilution and it is likely that pesticides would be absorbed through the skin differently from this residue. It is also possible that oral exposure can occur after contact with contaminated surfaces via 'hand-to-mouth activity'[13] but this pathway is secondary to dermal exposure and is not considered further here.

For each exposure scenario, a specific risk assessment needs to be carried out. This is based on a tiered system, whereby a first-tier assessment would use conservative default values and if the exposure is deemed to be too high, then higher tier assessments can be carried out that take specifics of the active ingredient (AI) and formulation into account. An exposure is deemed to be too high if it exceeds the Acceptable Operator Exposure Level (AOEL). An AOEL is assigned to each compound as a result of toxicological testing during its development, these values can vary greatly depending on the potency of the compound and its ability to cause adverse effects. The AOEL includes an uncertainty factor, typically at least 100, to allow for inter- and intra-species differences[8].

Each scenario has an associated methodology for calculating potential exposure. For example, in a dietary assessment, the total dose that a person is expected to consume is estimated by taking into account the level of pesticide residue in the product and the amount of that commodity consumed. A similar process exists for estimating dermal exposure, the external dose is first estimated and then an absorption factor is applied.

1.1.3.1 Worker Exposure Assessment

Estimation of the exposure of a re-entry worker is not an exact science, as the dose is not applied directly to the worker but is transferred from a contaminated surface. It is therefore necessary to estimate how much pesticide is on the contaminated surface and then to estimate how much of that will be subsequently transferred to the skin of the worker upon contact.

Assessment of re-entry worker exposure is based on the following equation for estimating Potential Dermal Exposure (PDE):

PDE (μg active substance/day) = DFR (μg/cm^2) x TC (cm^2/h) x T (h/day)

Where dislodgeable foliar residue (DFR) is the amount of substance upon a surface such as a leaf or fruit, that is available to be dislodged. DFR depends on application rate and efficiency as well as the concentration of the substance applied and the leaf area index. For a first-tier assessment, it is assumed that no dissipation occurs over time. If multiple applications are to take place, then accumulation of pesticide must be considered, in this case the DFR after the final application is used. This is estimated by taking in to account dissipation half-life for which the EFSA default value is 30 days in the absence of specific data.

In the absence of a measured value for DFR, a default of 3 $\mu g/cm^2$ per kg of AI applied per hectare (ha), is used. For example, if the application rate was 0.1 kg/ha, then the default value would be 0.3 $\mu g/cm^2$. This value is considered to be an over estimate and a worst-case scenario, if this value does not allow for an acceptable risk assessment then field studies to measure more realistic DFR values can be carried out.

The transfer coefficient (TC) is a measure of the intensity of contact with the contaminated surface. This value is dependent upon the type of crop and the task being performed[14, 15]. EUROPOEM II[16] values for TC are used for many different crop/task combinations; however, unfortunately the sources of these values are not publicly available. Generally, it is assumed that the worker will be wearing 'workwear' thereby having the torso, arms and legs covered and it is assumed that 10% absorption through this workwear will occur. Several studies have measured absorption of chemicals through clothing for potential dermal exposure[17, 18] and although this value is realistic for liquid forms of pesticide, this may not be the case for dried residues, that may not soak into materials and therefore could be less likely to penetrate them. Most of the contact with foliage will be via the hands, and so an obvious way to decrease exposure would be to wear gloves. However, it is not generally assumed that workers will be wearing gloves, as compliance is perceived to be low by regulators. This is due to many factors including a lack of understanding of potential dangers, and wearing

gloves while working in countries with higher ambient temperature can be uncomfortable. Furthermore, contrary to popular belief, gloves do not provide a complete barrier to exposure of liquid forms of pesticide, with breakthrough times ranging from 15 minutes to 24 hours[2, 4, 19, 20]. It is, however, possible to add a recommendation to wear gloves in a higher tier assessment if deemed necessary.

Time (T) is the amount of time in hours spent carrying out the task, default values for this are 2 hours for crop inspection and 8 hours for harvest.

The PDE is the total potential exposure of the worker; this value is given in μg per day and represents the external dose i.e., the amount of substance deposited on to the surface of the skin. To convert this into an estimate of the internal dose, here defined as that which is systemically absorbed, a percentage absorption factor must be applied. Default values can be used; the 2017 EFSA guidance on dermal absorption[10] updated these values to take formulation type in to account. For the concentrated products, this guidance recommends default absorption values of 25% and 10% respectively for organic-solvent based, and water-based/dispersed or solid products, respectively. For in-use dilutions, a default of 70% is recommended for organic-solvent based products and 50% for water-based/dispersed or solid. Generally, these default values are considered to be conservative and it is common for *in vitro* dermal absorption studies to be carried out to generate more realistic absorption values. These studies measure generally absorption from the concentrate and two relevant spray dilutions. The application that gives the highest percentage absorption value is then used. Conduct of these studies is explained in more detail in section **1.5**.

1.1.3.2 Limitations of the Current Approach

With respect to re-entry worker exposure, the use of these values may not be realistic. Previous work[21] has demonstrated that dermal absorption of dried residues may be different to that of a spray dilution and therefore the use of these

values for risk assessment may be inaccurate (discussed in section **1.6)**. McCarley et al[22] compared saturated aqueous solutions against solid active ingredient and found no significant difference in absorption into silicone membranes. However, a further study tested a similar hypothesis on human skin and found that the flux of the compound investigated was significantly lower from the powder than when in solution[23]. Although studies on neat active ingredient powders can give an insight into how a dried residue may behave, it would not always be the case that the residue remaining on a surface is completely dry. This would depend on the volatility of other components of the formulation.

In the case of a worker carrying out harvest, the exposure time is assumed to be 8 hours. The percentage absorption values are obtained from *in vitro* diffusion cell studies with an 8-hour exposure time i.e., a dose is applied at time 0 which is left in contact with the skin for an exposure period of 8 hours before a decontamination step is performed.

However, this is not representative of the exposure scenario. Although it may be the case that a worker is in the field for 8 hours in one day, the worker would not be exposed to the entire dose at the start of the day. In an example where an estimated exposure of the worker is 80 μg/cm^2/day, this worker would, in fact, be exposed to 10 μg/cm^2 each hour or 0.16 μg/cm^2 each minute and so on. This exposure varies in several ways to the scenario that is investigated *in vitro*. Assuming full decontamination occurs at the end of the working day, pesticide transferred from the first piece of foliage that the worker touches in a day (at time = 0) may have an exposure time of 8 hours. However, from then on, as time progresses, the exposure time is reduced. Taking this into account, and assuming a constant rate of exposure throughout the 8-hour working period; the average exposure time is in fact 4 hours. It is also unlikely that a worker would be in the field for 8 hours straight; it would be reasonable to assume that they may take a break to visit the toilet or to eat lunch, in which case it is also likely that they would wash their hands during this break. Another factor is that, after initial

exposure of an area of skin, contact with a second piece of contaminated foliage may not result in the same amount being transferred to the skin, or some of the original exposure could even be transferred back on to the foliage or fall to the ground. Equally, if the residue is in a form that is not well adhered to the skin e.g., solid crystals, just movement of the individual could cause the residue to be dislodged from the skin. It is unrealistic therefore, to assume that exposure throughout the working day is cumulative.

This project aims to investigate and address some of these inaccuracies, and develop a method where the dermal absorption of dried residues can be measured *in vitro* and compared against current practise, with the eventual aim of using this method to generate more realistic absorption values for re-entry worker assessment.

1.2 Structure and Function of the Skin

The skin is the body's largest organ, accounting for up to 10% of body mass[24-26]. It has a large surface area that is exposed to the external environment, averaging for an adult[24] around 1.7m^2. The primary function of the skin is to act as a barrier to both loss of internal water and uptake of xenobiotics from the environment. It also provides protection from mechanical stress and helps to regulate body temperature.

The structure of the skin can be considered as two distinct regions[27] (Figure 4) the dermis and the epidermis.

1.2.1 The Dermis

The dermis is 2-5mm thick[24]. It consists of mainly collagen and elastin fibres in an aqueous gel. It houses blood and lymph vessels which provide nutrients to and remove waste products from both the dermis and the avascular epidermis

above. Blood vessels extend as far as the dermo-epidermal junction where traversing molecules can then be absorbed systemically. The dermis also contains skin appendages which are discussed below.

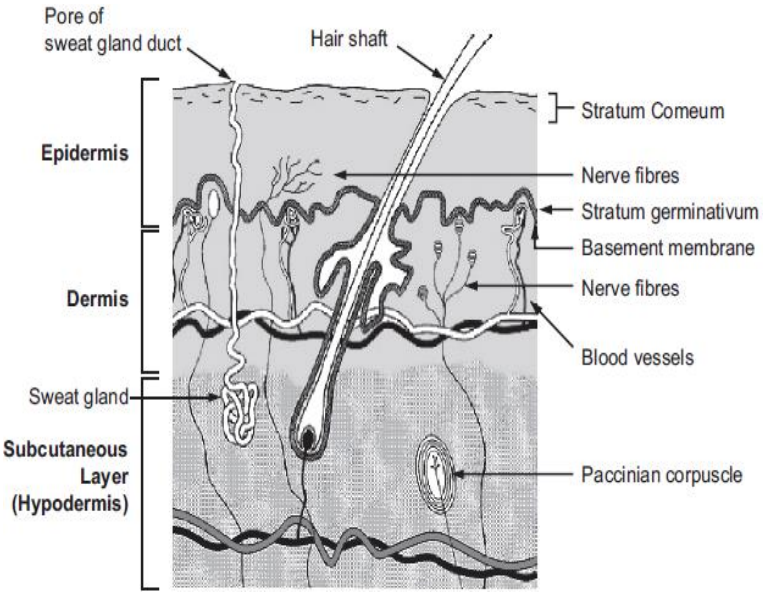

Figure 4 - Cross-sectional diagram of the skin [27]

1.2.2 The Epidermis

The epidermis is the outermost section of the skin and its depth varies from 0.06 to 0.8 mm depending on body site[24]. It is avascular and relies on the underlying dermis to sustain it. Figure 5 shows the different layers of the epidermis, the innermost of which is the *stratum basale* or basal layer. The most abundant cell type in this layer are the keratinocytes. These cells are the only epidermal keratinocytes capable of undergoing mitosis; upon replication, daughter cells move towards the surface, differentiating to form the outer layers of the epidermis. The basal layer also contains several other cell types. Langerhans cells are dendritic antigen-presenting cells that may be important in allergic contact dermatitis[28]. Merkel cells are thoughts to be involved in touch sensation. Melanocytes are pigment forming cells, they produce eumelanin (brown) and

phaeomelanin (red) which contribute to the skin's colour and protects from UV damage.

The layer that lies above the basal layer is the *stratum spinosum*, which gains its name from the desmosomes that link the cells, appearing like 'spines'. As the cells migrate outwards, they become more keratinised, flattened and begin to lose some organelles, forming the *stratum granulosum*. Cells within this layer begin to synthesise granules of keratohyalin which are thought to be involved in cell keratinisation and 'membrane coating granules' which release lipids into the intercellular space by exocytosis[29, 30]; the significance of these lipids is discussed in more detail below.

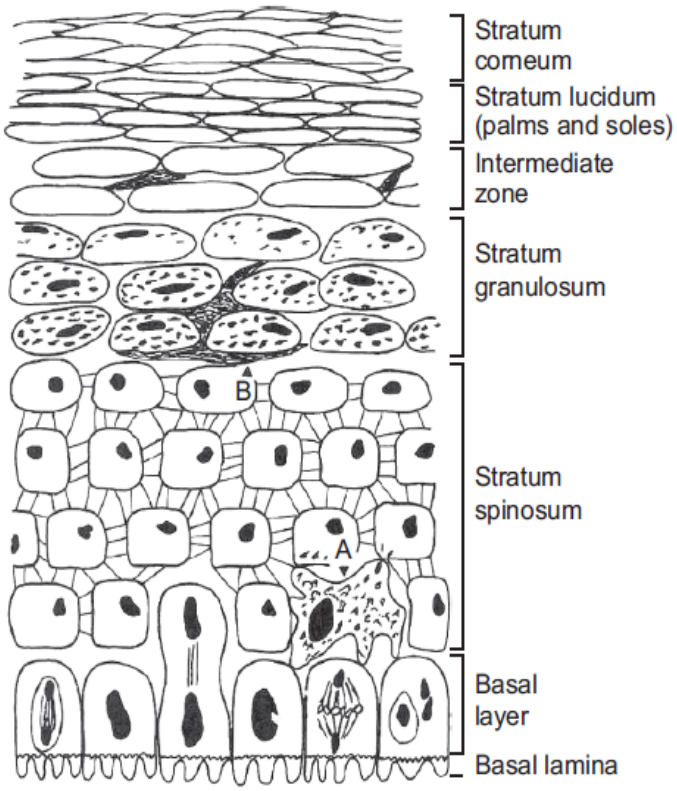

Figure 5 - A schematic of the epidermis [1]

1.2.3 The Stratum Corneum

The stratum corneum (SC) is the outermost layer of the epidermis. It is terminally differentiated and consists of non-viable, flattened, keratin-packed cells with no nuclei. It is generally only around 10-15 cells thick, giving it a depth under normal conditions of around 10 μm[24]. Nonetheless, the SC is responsible for most of the skin's barrier function. When it is removed, or damaged by disease, trans epidermal water loss increases by an order of magnitude[31-33] and xenobiotics may pass into the body with greater ease[34]. The SC is often described in terms of a 'bricks and mortar' model. The bricks represent the keratin packed cells described above. The mortar represents the lipids surrounding the cells; which are instrumental to the barrier function of the skin[35]. These lipids have a unique composition, consisting of large amounts of ceramides and free fatty acids[26, 27, 30]. Other components include cholesterol, cholesterol sulphate, glucosylceramides and sterol/wax esters. The organisation of these lipids is important; a highly ordered system of bilayers is formed[36, 37] which is responsible for the efficiency of the barrier. Disruptions to the SC lipids by disease[38] or by solvent extraction[33, 39] have been shown to cause compromised barrier function. Routes of permeation through the SC are discussed in section **1.4**. As cells move towards the outer layers of the SC, desmosomes holding the cells together begin to break down causing their loss from the skin's surface, known as desquamation. The entire SC is turned over in around 2-3 weeks[40].

1.2.4 Skin Appendages

Human skin has four types of appendage, hair follicles, sebaceous glands, eccrine sweat glands and apocrine sweat glands. Hair follicles can be found on all areas of the skin at differing densities other than the palms, soles and lips. Sebaceous glands are most abundant on the forehead and secrete sebum which serves to regulate surface pH and keep the skin moist[2]. Eccrine sweat glands secrete sweat

and help regulate body temperature, and are found across most of the skin surface. Apocrine sweat glands are larger, localised around the arm pit and genital regions[27], and respond to emotional stimuli. They secrete a mixture of proteins and lipids that upon breakdown by bacteria is responsible for the odour associated with sweat[41].

1.3 Physiological Factors Affecting Dermal Absorption

The effect of ageing of the skin on dermal absorption is not well studied. In general, with ageing, the skin becomes more dry[42] and the depth of the SC increases, which would suggest an increase in barrier function. One study found absorption to be significantly lower in aged skin and that this effect may be more pronounced for hydrophilic molecules[43]. It is thought that changes in hydration, blood flow and lipid composition could be responsible for changes in barrier function[44]. Some of the changes may not be a direct result of 'ageing' but due to a lifetime of UV exposure[45]. Skin damage caused by UV light has been shown to adversely affect barrier function[46], this could be a relevant factor for re-entry workers who will often spend most of their day outside in the sun.

Dermal absorption varies with anatomical site[47-49]. The SC is thickest at areas that are frequently exposed to mechanical stress such as the palms of the hands and soles of the feet. Permeability is greatest through scrotal skin which has a thin SC and high density of hair follicles[48]. This regional variation is of potential relevance to re-entry workers because most exposure is via the palms of the hands, where the skin is less permeable than the torso for example. Conversely, the forehead is an area of high permeability that may also be exposed during re-entry activities.

Blood flow to the skin is controlled by the sympathetic nervous system in response to changes in core temperature[50]. Increased blood flow serves to increase heat loss and consequently decrease core temperature. Re-entry workers may spend long periods of time outdoors carrying out manual tasks in warm

ambient temperatures; as the optimal time for harvest is usually in the summer. Cutaneous blood flow has been shown to increase with increasing temperature[51, 52] and in response to excercise[53]. This increased blood flow could influence permeation[54]. The activities of the re-entry worker may also increase levels of perspiration. An extreme case could mean that the worker has sweat on the surface of the skin, which could affect the amount of pesticide that is transferred to the skin. This could also be significant when exposure is to a dried residue as the sweat may also provide a vehicle in which a dried residue can dissolve, possibly resulting in enhanced absorption. In a less extreme case, the SC could be more hydrated than it would be under normal conditions. Increased hydration of the skin can cause permeation to increase by an order of magnitude[55]. Similarly, occlusion of the skin has been shown to affect absorption[56]. This excess hydration can disrupt SC lipid structure and organisation by introducing 'pools' of water in the intercellular space[55-57].

Since it is the outer layer of the skin that provides much of the barrier function, seemingly superficial damage to the skin can represent serious damage to the SC and this can result in a compromised barrier function[34, 58]. Damage to the skin may be relevant to a re-entry worker as abrasion of the skin could occur as part of their daily work (e.g., scratches by branches or the skin pierced by thorns), although protective gloves are typically worn where this is expected.

Occupationally related skin diseases are common[2, 59, 60] especially when the skin is exposed to a humid environment for extended periods of time. Many of these diseases such as atopic dermatitis can compromise the skin barrier and dermal absorption can be increased[58, 61].

The skin has some metabolic activity, including cytochrome P450 and non-specific esterase enzymes which reside in the viable epidermis. These enzymes can metabolise molecules and consequently reduce their absorption[2] such as some insecticides[62]. However, this metabolism is not always protective in nature.

One of the best studied incidence of skin metabolism is that of polycyclic aromatic hydrocarbons which are not themselves especially toxic, but can be activated in the skin, these metabolites can then bind to DNA and can cause cancer[40]. Skin used for dermal absorption studies *in vitro* is generally not metabolically active. This can potentially cause some disparity from the *in vivo* scenario, but this would usually result in an overestimate of absorption due to lack of metabolism[9].

1.4 Skin Permeation

The ability of molecules to be absorbed into and across the skin is of significance, both in relation to occupational exposure as described above, and in relation to the delivery of drugs through the skin for medical purposes. Although absorption into the skin tissue can cause local toxicity, and is important when considering locally acting creams and cosmetics, this document will focus mainly on permeation of molecules through the skin into systemic circulation as this is generally the greatest source of toxicity.

For a molecule to be systemically absorbed, it must permeate to the dermis where it can then pass into the vasculature. Dermal penetration is expressed in terms of flux (J); the mass of compound traversing a membrane per unit area per unit time.

Despite being a heterogeneous membrane with several different permeation pathways, it is possible to describe permeation through the skin in terms of simple kinetic equations. These equations are useful to aid understanding of how the diffusion process works and can also be used to predict the behaviour of a molecule applied to the skin.

The maximum flux J_{max} of a substance across the skin at steady-state can be estimated using Fick's first law of diffusion (Equation 1)

$$J_{max} = k_p \cdot C_{sat} \qquad \textit{Equation 1}$$

Where k_p is the permeability coefficient (cm/h) of the compound across the skin from a simple aqueous vehicle and C_{sat} is the chemical's solubility in water. According to Fick's first law, the concentration gradient across the membrane is directly proportional to the flux. If we assume sink conditions, i.e., the dissolution of the compound in the plasma or receptor solution is not a limiting factor, then the concentration on the receptor side of the membrane is effectively zero. Therefore, the concentration gradient can simply be replaced by the applied concentration. k_p is dependent on the molecule's diffusivity (D), partition coefficient ($k_{sc/v}$) and the path length (h) (Equation 2)

$$k_p = \frac{D \cdot k_{sc/v}}{h} \qquad \textit{Equation 2}$$

The first step in absorption is partitioning of the molecule into the SC. The partition coefficient is dependent upon a molecule's relative affinity for its vehicle and the SC. For example, a lipophilic molecule presented to the skin in a lipid-based vehicle would be less likely to partition into the SC than if presented in an aqueous vehicle. Similarly, a hydrophilic molecule would show a higher affinity for an aqueous vehicle than the lipoidal SC. In the case of an aqueous vehicle the partition coefficient can be approximated using the octanol/water partition coefficient (P).

Using this and the molecular weight (MW) of the compound, an algorithm developed by Potts and Guy[63], using a database of experimentally determined fluxes, can be used to estimate k_p (cm/h) (Equation 3).

$$log\ k_P = -2.7 + 0.71 \cdot log\ P - 0.0061 \cdot MW \qquad \textit{Equation 3}$$

where P is the chemical's octanol-water partition coefficient and MW its molecular weight. This equation predicts that the permeability of a molecule will increase with decreasing MW, as is commonly the case for passive diffusion, due to decreased resistance being associated with decreased molecular size. Also, it indicates that permeability increases with increasing lipophilicity. This is true for moderately lipophilic compound for which the SC is the main barrier to absorption. However, for more lipophilic compounds the viable epidermis could present more of a barrier, and partitioning into the viable epidermis from the SC may become rate limiting. ${k_p}^{corr}$ described in Equation 4 is a corrected permeability coefficient to take this into account[64] :

$${k_p}^{corr} = \frac{k_p}{(1+\frac{\{k_p \cdot \sqrt{MW}\}}{2.6})} \qquad \textit{Equation 4}$$

Therefore:

$$J_{max} = \frac{k_p}{(1+\frac{\{k_p \cdot \sqrt{MW}\}}{2.6})} \cdot C_{sat} \qquad \textit{Equation 5}$$

The equations above describe absorption of molecules from an infinite dose of a saturated aqueous solution. In the case of drugs being delivered to the skin from a transdermal patch, this model is reasonable and it may be possible to achieve

maximum flux. However, it is rarely the case that a molecule is presented to the skin in such a way. Formulations of pesticides and medicines often contain other solvents, and ingredients such as emulsifiers. Therefore, partitioning is more complex and difficult to predict. An increase in a compound's solubility in its vehicle will generally result in a decrease in its partition coefficient and therefore, a decrease in flux. Paradoxically, the flux of a compound is directly proportional to its concentration in the vehicle. In the case of exposure to pesticide spray dilutions, the compound of interest would often be significantly below its saturation concentration in the vehicle and therefore maximum flux would never be achieved.

On the other hand, in a case where the molecule is applied to the skin as a suspension, the solution may be saturated at the time of application, but solubilisation of undissolved material into the vehicle could then become the rate-limiting step in absorption. The impact that a vehicle can have upon absorption is recognised by EFSA[7] in that if the constituents of a formulation are changed by at least 25% w/v, then new dermal absorption values must be obtained for risk assessment. This guidance has been updated in the 2017 document[10] where a more complex system taking into account the concentration of the excipients is being implemented.

Finally, exposure to chemicals such as pesticides is generally in the form of a finite dose. Therefore, the driving force for absorption is greatest at the time of application and tails off as the dose depletes over time. Cumulative penetration (Figure 6) from a finite dose would increase rapidly after the initial lag time and then level out. However, from an infinite dose the cumulative permeation would theoretically increase indefinitely.

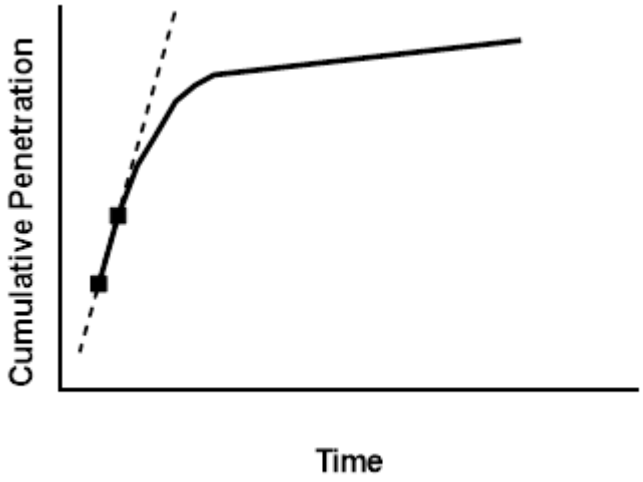

Figure 6 - A model graph of cumulative penetration against time for (a) infinite dose (dashed lined) (b) finite dose (solid line)

There is a grey area between a true 'infinite dose' and a 'finite dose' where the absorption of a compound is 'flux-limited'. This type of exposure is seen regularly in the literature and its significance is often overlooked. Several recent critical reviews have outlined the importance of identifying a flux-limited scenario and how this can affect percentage absorption results[65, 66]. Kissel[65] describes a dimensionless absorption value that could be useful in determining if a set of exposure conditions represents a flux-limited dose. In a flux-limited scenario, fractional absorption is inversely related to loading[67] i.e., the higher the loading, the smaller the fraction absorbed. This is particularly important for risk assessment because it is common to use percentage absorption factors. If the experiment where the percentage absorption value was obtained has relevant exposure conditions i.e., application of a similar skin loading from a relevant formulation, then this is an acceptable approach. However, the use of the same percentage absorption value to describe other exposures to the compound may not be appropriate. Additionally, from finite doses, binding to components in the skin, and reservoir accumulation in the SC, can have a more significant impact on the fraction absorbed.

Potential routes across the SC are transcellular, intercellular or appendageal (Figure 7). Although there are theoretically three different pathways across the SC, absorption of an applied chemical will most likely be via a combination of these three pathways. The physicochemical properties of the molecule in question will affect which pathways are most dominant.

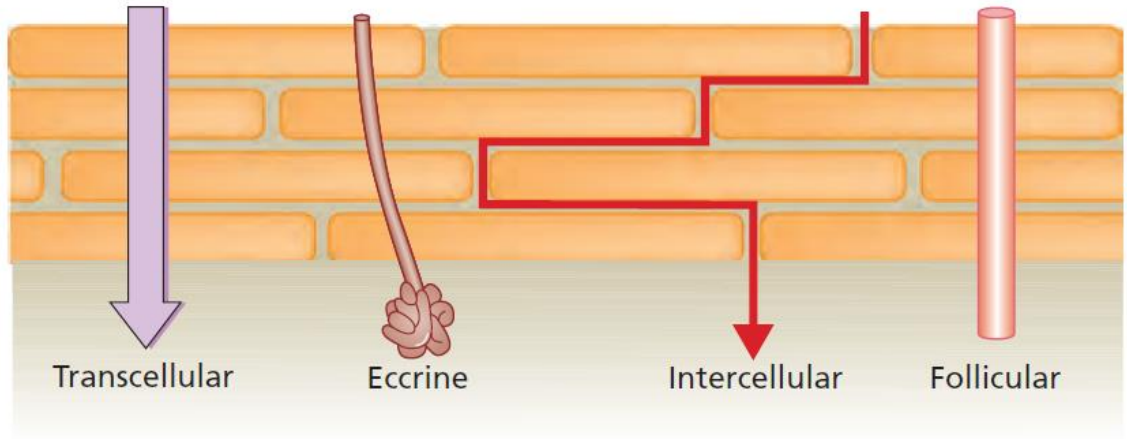

Figure 7 - The bricks and mortar model and pathways across the stratum corneum [68]

As described above, the keratinocytes of the SC are surrounded by lipid. Therefore compounds that are able to dissolve in both aqueous and lipid phases (log P ~ 1-3) generally permeate more effectively[69]. The theoretical path length of a molecule diffusing the SC has been calculated as around 50 times the SC depth[60], suggesting that the molecules take a tortuous route around the corneocytes. It is generally thought that passage through this lipid phase represents the major pathway for most compounds through the SC[30, 70] apart from those that are very hydrophilic. It may be possible for more hydrophilic molecules to partition into the keratin packed cells. However, this route is not continuous, the molecule would still be required to partition in to the intercellular lipids between each cell to traverse the SC.

The appendageal route is not generally thought to be important at steady-state in humans due to their relatively low density, only representing from 0.1 – 0.2 %

of the total surface area[71]. However, it may be more important in transient exposure scenarios, in the early stages of absorption[72] and for hydrophilic molecules[73-75]. Several studies[76, 77] have demonstrated the importance of the follicular route in the absorption of the hydrophilic compound caffeine. When hair follicles were artificially blocked, caffeine detection in plasma was delayed, suggesting that the follicular route provides a faster route through the skin. Another study using Stimulated Raman Scattering (SRS) found that the penetration of polyethylene glycol (PG) via a hair follicle was rapid compared to penetration via the SC lipids[78].

Once a molecule has traversed the SC it must then partition into the viable epidermis which is much more hydrated. Compounds that are particularly lipophilic may partition into the SC easily but partitioning into the more aqueous lower epidermis becomes the rate-limiting step[79]. As a compound cannot be absorbed systemically until it has traversed to the dermis these very lipophilic compounds are often poorly systemically absorbed. This can create a 'reservoir' whereby the lipophilic compound accumulates in the SC from which it is then slowly released into the viable tissue and circulation[80]. Depending on the rate of removal of this compound from the reservoir, it could also potentially be lost via desquamation. Although, due to the relatively slow rate of epidermal turnover, this is only likely to be significant for compounds with very slow absorption[81].

1.5 Measuring Dermal Absorption

Ideally, in order to gain the most realistic results, dermal absorption for each product would be measured in humans *in vivo* using relevant exposure conditions. Although this may frequently be possible for pharmaceuticals, it is often deemed unethical to deliberately expose humans to pesticides, due to toxicity concerns and the fact that, unlike pharmaceuticals, most pesticides are not designed for dosing humans.

In the absence of *in vivo* human studies, *in vivo* animal testing may be considered. Measurement of dermal absorption *in vivo* is most often carried out in rats; this is due to their use being relatively inexpensive, ease of handling, and the existence of a well-defined protocol[82-84]. It is also possible to use hairless mice and guinea pigs for *in vivo* investigation[85]. However, as mentioned above, the permeability of skin varies between species and therefore the use of human skin *ex-vivo* may give a better estimation. One method that attempts to address this is the 'triple pack' approach[9, 86]. This method carries out *in vivo* and *in vitro* rat as well as *in vitro* human studies. The two *in vitro* results are compared in order to assess the difference in absorption between the species. This difference is then used to extrapolate the *in vivo* rat results and estimate *in vivo* human absorption.

Methods for measuring dermal absorption *in vitro* are widely used. As the SC is the main barrier to absorption, skin maintains its barrier function *ex-vivo*. Several studies have shown that trans-epidermal water loss (a good measure for barrier function) is similar *in vivo* and *in vitro*[87, 88] and that *in vitro* permeation is generally a close estimate of *in vivo* permeation[89].

1.5.1 *In vitro* diffusion cell testing

To design a protocol, many decisions need to be made such as the type of skin to be used, the type of diffusion cell, which receptor solution, and many more. In an attempt to standardise the conduct of *in vitro* diffusion testing between labs, specific guidance is given in OECD 428[90], with more specific guidance for dermal absorption studies for pesticide risk assessment by EFSA[8, 10]. When the absorption of model compounds has been compared between labs, they have been shown to give similar results[91]. Also, it has been shown that *in vitro* absorption measurements give a good approximation of *in vivo* absorption[86, 92, 93].

1.5.1.1 Sources of Skin

It is preferable to use human skin for risk assessment. Human skin can be obtained from cosmetic surgery, skin banks or cadavers. However, the use of human skin can be expensive and logistically difficult. It is often unknown exactly how the skin has been treated before it is received. For example, if the skin is from surgery it is common that scrubbing with alcohol will have occurred prior to excision. The pieces of skin are also often small and irregularly shaped, which can make handling and preparation difficult.

For these reasons, it is sometimes preferable to use the skin of other species. Many animal skins have been tested including rat, hairless mouse, guinea pig, snake and dog[2, 24]. However, absorption of chemicals varies between species[2, 9] due to variations in follicular density, SC structure and lipid composition. Rat skin is commonly used both *in vivo* and *in vitro* but has been shown to be more permeable[91] than human skin. Pig skin is thought to be a good model for human skin as it has a similar lipid composition[94] and follicle density[95] and studies have demonstrated similar absorption to human skin[96, 97].

The advantages of using pig skin are that the investigator controls what happens to the skin post-mortem, meaning it is less likely to be damaged. Also, the fact that the skin received from sacrificing one pig has an area of around $1m^2$ means that handling is much easier, as it can then be cut in to sections of the desired size. Additionally, multiple replicates can be taken using the same donor, therefore eliminating inter-individual variability. This can be useful in scientific investigations where the difference between two outcomes is small and may be masked by inter-individual variability. However, if the aim of the study is to gain an absolute value of absorption, more than one donor should be used. EFSA guidance[8] recommends the use of skin from at least 4 donors. This is to provide a more representative sample of the population.

1.5.1.2 Preparation of Skin

Skin used in dermal absorption studies is usually not full thickness. The use of full thickness skin often results in lower levels of the test compound in the receptor solution[98] and increased lag times. This is because a molecule must traverse the hypo-dermis before reaching the receptor solution, which would not be necessary to be absorbed systemically *in vivo.*

Probably the most commonly used is split-thickness or dermatomed skin. A dermatome is an instrument that can cut the skin to a nominal depth that would usually include the epidermis and part of the dermis.

Another common method for preparing the skin is by heat separation[99], the process of submerging the skin in water at 60°C. This causes the epidermis to separate from the dermis without significantly impairing the barrier function. The upper layers can then be used for diffusion studies. SC can also be isolated by trypsin digest, but is very fragile due to being extremely thin and will result in the SC being unrealistically hydrated[27].

After the skin has been prepared, it is common to freeze it for later use, it is generally accepted that barrier function is not affected by this process[100]. If the skin is to be used for regulatory studies then tests are performed to determine the integrity of the barrier. When using human skin, as described above, small pieces of skin that come from a surgical environment can easily be damaged. Methods to measure barrier integrity include trans-epidermal water loss[34, 101], capacitance[102] or diffusion of tritiated water[90].

1.5.1.3 Diffusion Cells

Once the membrane has been selected and prepared, *in vitro* dermal absorption studies can be carried out using diffusion cells. The most common type of cell was designed by Franz in 1975, who found a good agreement between *in vitro*

and *in vivo* absorption of 12 compounds[92]. Figure 8 shows a static Franz diffusion cell, an apparatus usually made of glass, and within which a piece of skin can be secured. On the upper side of the skin is the donor chamber, where the product to be tested is applied, on the underside is the receptor solution which mimics plasma. The receptor solution is stirred with a magnetic bar and is normally maintained at 37°C, with the aim of achieving a skin surface temperature of 32°C, the average temperature of the skin surface *in vivo* at rest.

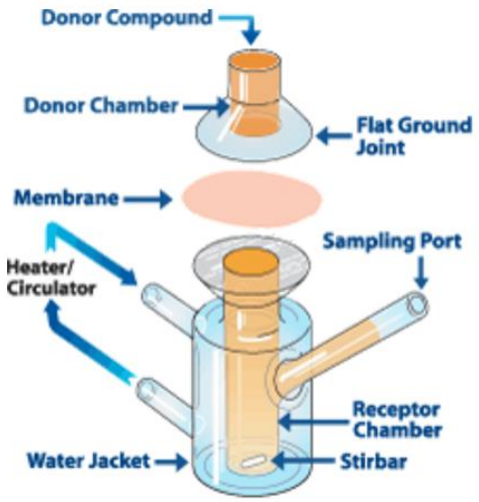

Figure 8 - A Franz diffusion cell. [103]

Another common cell type is the 'flow through' diffusion cell. The receptor solution beneath is constantly replaced, with the aim of mimicking blood flow. However, generally no differences in the ability of the two cell types to predict the *in vivo* scenario have been found[91, 104, 105] and therefore static Franz cells are usually preferred due to the experimental setup being significantly less complicated.

1.5.1.4 Receptor Solution

Any molecule that passes through the skin must then dissolve into the receptor solution. The receptor is sampled at various time points to provide an estimate of the amount that would be systemically absorbed. The most common receptor

solution is phosphate buffered saline (PBS) at pH 7.4. It is essential that 'sink conditions' are maintained i.e., the dissolution of the compound in to the receptor solution is not rate limiting (solubility in the receptor solution should be at least 10x higher than the highest observed concentration[90]). For compounds with low aqueous solubility this can be achieved by altering the receptor solution to increase its solubilisation capacity. The most common methods for achieving this are to include up to 6% Volpo, a non-ionic surfactant, or 5% bovine serum albumin[106]. Some studies have used a receptor solution of 50:50 ethanol:water to increase solubility of the test compound; however, the use of organic solvents is not recommended as this can alter the barrier properties of the skin[107].

1.5.1.5 Exposure Type

It is important to replicate the exposure that is being investigated as closely as possible. The use of a relevant dose and a commercially relevant formulation are both important. Although the physicochemical properties of the compound of interest will have a large effect upon absorption, this can be modulated by the vehicle in which it is presented to the skin. It is important that the application is from a vehicle with relevant components. It has been shown that pesticides presented to the skin in commercial formulations may be absorbed more efficiently than in an aqueous donor[108] or acetone vehicle [109]. This is because these formulations often contain solvents which may enhance penetration through the skin. This can occur when the solvent passes into the skin and carries the solute with it[110, 111] or when the solvent actively damages the barrier function of the skin[107, 112, 113]

It is also very common for commercial pesticide formulations to contain one or more surfactants. Surfactants are often added to lower the surface tension of the solution which can improve spreading and wetting, and decrease the amount of substance that needs to be applied. Surfactants can also increase mixing between hydrophilic and lipophilic substances; this property is useful to enhance

penetration of hydrophilic substances into the wax of a leaf, for example. Due to these very properties, surfactants can enhance penetration of substances across the skin[114-117].

1.5.1.6 Wash Procedure

At the end of the prescribed exposure time, the skin is decontaminated. Depending upon the aims of the study, sampling may continue after this time point. In risk assessment, the wash procedure typically uses an aqueous soap solution to replicate a worker washing their hands at the end of a working day. However, when wash efficiencies have been measured, some studies have found that washing immediately after exposure did not remove all pesticide[118, 119] and that the lipophilicity and solubility of the compound can affect wash off efficiency[2]. This could be due to the short window of time that is available for the wash solution to solubilise the compound[120, 121].

1.5.1.7 Tape Stripping

Tape stripping is a method used both *in vivo* and *in vitro* for measuring the distribution of a molecule within the SC[122]. This procedure is generally carried out upon termination of the experiment and consists of applying adhesive tapes to the surface of the skin and removing them, thereby removing a layer of SC cells. It is also possible to weigh the tapes before and after the tape stripping process in order to estimate the amount of SC that has been removed. These tapes are then extracted and quantified for the molecule of interest. Using a combination of the estimated mass of SC and the concentrations of the molecule within, a depth profile can be plotted. Although it is not clear if all of the SC removed with each tape is from the same depth, as furrows in the skin can cause variation[123]. Regardless, tape stripping is a powerful method that is simple and inexpensive.

In the case of studies for drug delivery, it is common to assume that any drug recovered in the tapes is absorbed. However, in studies carried out for regulatory approval of pesticides in the EU it is common practice to assume that any substance found in the first two tape strips is not absorbed[8] but will instead be lost via desquamation. If particularly lipophilic (log P > 3) then it may be reasonable to assume that the molecule would stay within the SC long enough to be lost via desquamation. However, for moderately lipophilic or hydrophilic compounds this assumption may not be true[81] and in fact the SC can act as a reservoir from which the compound continues to be absorbed after the skin has been decontaminated[69]. One study by Zendzian et al showed 17 out of 19 compounds continued to be absorbed after skin decontamination[124], a reservoir remaining in the SC was postulated to be the cause.

1.6 Absorption of Pesticides from Dried Residues

As described above, exposure is not always to a solution, but can sometimes be to a solid or residue. To measure dermal absorption, EFSA guidance on dermal absorption[8] recommends that 'solid material should be moistened with a minimal volume of vehicle to make a paste' or occlusive conditions used. This is because there is currently no defined methodology for application of solids in dermal risk assessment. Upon application of a finite dose of solid powder, it is unclear how much of the skin surface is covered, so moistening the solid would likely increase the area of contact. Moistening or using occlusive conditions could also mimic sweat on the surface of the skin. However, skin mounted in diffusion cells *in vitro* may already represent an over-hydrated system, which would represent a worst-case scenario.

Previous work[21] carried out in Bath designed an *in vitro* method to measure absorption of pesticides from dried residues. The active ingredient (AI) was mixed with acetonitrile to form a solution or suspension. This was applied by

spin coating to a steel disc and allowed to dry to form a residue. The disc was then placed on the skin surface and left in contact for an 8-hour exposure period (Figure 9).

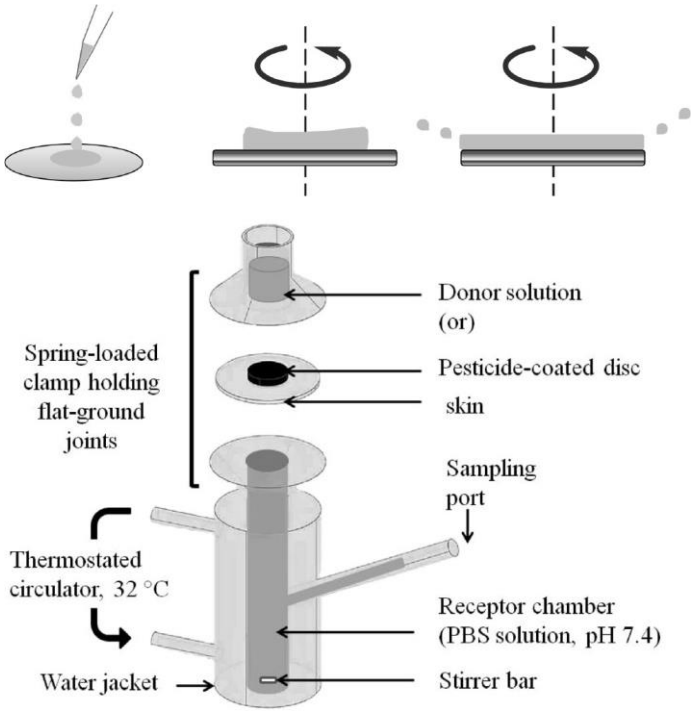

Figure 9 - Experimental set-up used by Belsey et al[21], steel discs spin coated with pesticide applied to skin mounted in a Franz diffusion cell.

This work showed that pesticides were absorbed differently when applied as a residue than when the same compound was applied as a solution or suspension. Four of the six compounds investigated were absorbed significantly less when applied as a residue, one compound showed no significant difference, and one compound was absorbed significantly more when applied as a residue.

However, the methods used had several limitations that call in to question whether the results are entirely representative of the exposure scenario.

Formulation effects were not taken into account. Pesticide products come in many different formulations, often containing other solvents and surfactants. If these other excipients are non-volatile, then they form part of the residue to which a worker is exposed and may therefore have an effect on the dermal

absorption of the AI. It would be more realistic to measure AI absorption from dried residues derived from commercially relevant formulations.

The two dose levels used were 1000 μg/cm^2 and 100 μg/cm^2, the lower of these is at the upper limit of a realistic exposure. It is possible that absorption is flux-limited for some compounds at this dose level. As described in section **1.4**, fractional absorption can be dose-dependent and therefore lower doses more relevant to a typical exposure should be investigated.

Spin coating was used to apply the pesticide in an attempt to achieve a uniform covering across the disc. However, this resulted in up to ±15% uncertainty in the amount of AI applied. If it is not possible to confidently state the amount originally applied, this reduces confidence in the results.

The diameter of the disc was 12 mm (area of 1.13 cm^2) while the area of skin 2cm^2, meaning only about 60% of the skin surface was covered by the disc. Although this was taken in to account in the analysis by expressing absorption per square centimetre, it is possible that lateral diffusion occurred. Also, it was not clear how much of the disc was in direct contact with the skin, due to the presence of hairs, furrows and crevasses. There is uncertainty, therefore about the surface area of skin that was exposed.

Finally, leaving the disc in contact with the skin for the duration of exposure, created occlusive conditions which may not be present in an actual exposure scenario.

1.7 Aims

The aim here, therefore, was to develop and validate a more realistic *in vitro* method for quantifying the dermal absorption of pesticides from dried residues that satisfied the following criteria:

- Exposure under non-occlusive conditions.
- Quantification of the amount of residue applied to the skin.
- Use of commercially relevant formulations.
- Applying doses relevant to re-entry workers.

It was necessary, therefore, to a) create a dried pesticide residue, and b) transfer this residue to the surface of the skin *in vitro* in a quantifiable manner.

2 METHODOLOGY

2.1 Method Development

Development of the methodology described in this chapter is not presented in chronological order, but rather split into three main decisions. The nature of method development means that none of these decisions could be made independently.

2.1.1 Surface

Belsey et al tested many types of artificial surface for uniformity of residue coverage after spin coating: glass coverslips, acetate transparency film, steel atomic force microscopy (AFM) discs and a selection of polyethyleneterephthalate (PET), which were uncoated, aluminiumised, or siliconised. Steel AFM discs were chosen due to their 'ease of handling, regular shape, and superior residue coverage'[21].

2.1.1.1 Wheat Leaves

An initial aim of the present project was to investigate the possibility of using a leaf surface (as opposed to a steel disc) in the method i.e., one more representative of a re-entry worker exposure scenario.

However, the properties of leaf surfaces vary greatly between species, age of the plant and time of year[125]. Also, exposure may occur from other parts of the plant such as fruit, which will have very different surfaces. Therefore, the

choice of a 'representative' plant surface is not straightforward. First of all, to test the viability of using a leaf surface, wheat was chosen as a model; wheat is a very common crop, with 16.1 million tonnes being produced in the UK alone in 2015[126]. Wheat can also be grown fairly quickly and easily in a greenhouse.

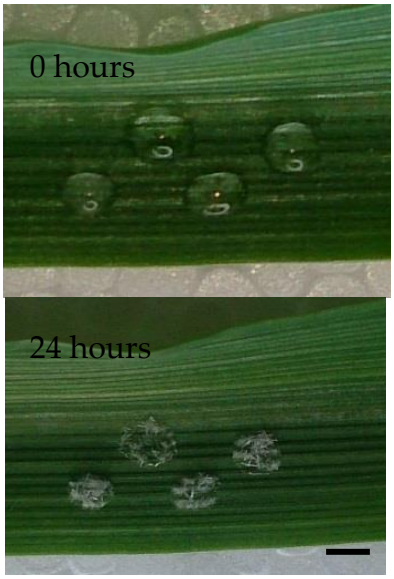

Figure 10 - 0 hours) Atrazine in methanol applied to a wheat leaf. 24 hours) Atrazine crystals remaining on the leaf after evaporation of the solvent. Scale bar = 2mm

Using seeds provided by Syngenta, wheat was grown in a greenhouse at the University of Bath and leaves were used after 10 weeks.

Four 1 μL droplets of atrazine (20 mg/ml in methanol) were pipetted onto a wheat leaf, still attached to the living plant, and were allowed to dry for 24 hours. Application methods are explained further in section 2.1.2. Figure 10 shows an image of the droplets immediately after application and 24 hours later when the solvent has evaporated away, leaving a 'residue'.

A potential issue when using leaves is that many pesticides are designed to translocate to different parts of the plant to elicit their effects. Therefore, it is

probable that after 24 hours, less than 100% of the applied AI would be available on the surface of the leaf. To test for translocation of atrazine, leaf sections were extracted in methanol 24 and 72 hours post-application. The mean recoveries from an 80 μg application were 81.2 ± 2.5 μg and 80.9 ± 1.3 μg for 24 and 72 hours respectively (n=5). This demonstrates that the atrazine has not moved away from that part of the leaf, however it does not give information about whether the atrazine has permeated into the leaf wax or into the plant cells themselves. With a log P of 2.7 it is likely that some of the atrazine has partitioned into the lipids of the leaf wax. To investigate this, a scalpel was used to scrape the residue remaining on the surface of the leaf after 24 hours into a vial. Although every effort was taken to only remove residue on the surface, to make sure all residue was removed, it is possible that some leaf wax was also removed. When quantified only 61.5 ± 9.6 μg of the 80 μg applied were available, final recovery was 98 ± 5.8 % after extracting the remaining amount in the leaves (n = 10), this suggests that around 25% of the atrazine has passed into the leaf. Also, the amount recovered was much more variable, with highest and lowest recoveries of 71.3 μg and 48.2 μg respectively, although this could be an artefact of the removal process. This uncertainty about the exact amount of pesticide remaining on the surface of the leaf means that it would be difficult to accurately quantify the amount applied to the skin following transfer. In addition, experiments where a leaf was rubbed against a piece of filter paper to measure the transfer of atrazine left green marks on the filter paper. This suggests that not only the atrazine was being transferred, but part of the leaf also.

2.1.1.2 Inert Disc Coated in Wheat Wax

To simulate the surface of a leaf, the aim was to use wax extracted from wheat, (supplied by the University of York) to create an inert surface onto which pesticide droplets would settle and dry in a similar manner to that on a leaf surface. This surface should have a smooth, even layer of wax similar to that found on the corresponding leaves.

Various solvents were tested for their ability to solubilise the wax and it was found that concentrations up to 3% w/v could be achieved in toluene. 50 μL of this solution was then spin coated onto various platforms (glass cover slip, aluminium disc, siliconised polyethylene terephthalate (PET)).

Figure 11 - Wheat wax on a 12mm glass disc

However, spin coating left an uneven surface, often producing a circle of wax around the outside of the disc but with none in the middle. It was concluded that this may be due to the high boiling point of toluene which therefore was not evaporating away quickly enough during the spin coating process. After trying various ratios of methanol, ethanol and toluene, it was found that a 3:1 mixture of methanol to toluene, warmed to 60^0C before application, gave the smoothest surface upon spin coating.

This solution was also pipetted directly onto the disc and allowed to evaporate without spin coating Figure 11. Under inspection with a light microscope, no visual differences in coating consistency were observed between these two methods.

To compare residue consistency between the wax surface and a leaf, droplets of pesticide were applied to the treated disc and to a wheat leaf. Upon visual comparison it was evident that the wax disc was not a good model for the leaf. Liquid on the leaf beaded and formed a droplet (Figure 10) but droplets on the wax-treated disc 'sank' into the surface and did not form distinct droplets. It was not possible, therefore, to produce a dried residue of pesticide on the surface of the wax disc, presumably because the complex structure of wax on the plant[125] is not mimicked well by the model.

2.1.2 Application

While Belsey et al used a spin coating technique to apply pesticide to discs, this approach is not representative of how a residue would be formed in the field. It is also not easily reproducible; specialist equipment is required and spin speeds and times have to be optimized for each compound (and presumably each formulation) to achieve acceptable results.

Figure 12 - A spray bottle mounted above a glass disc ready for application of a pesticide solution.

The most common method of pesticide application is in the form of a spray. This may involve a hand-held sprayer/knapsack or a tractor mounted spray boom. These methods are obviously inappropriate for an *in vitro* laboratory setting and other more appropriate methods for applying pesticide to the platform were therefore investigated.

A small throat spray bottle marketed as a 'metered dose system' was obtained (Cavonia, Thornton and Ross Limited, UK). This bottle was pump action and had a screw off lid so that the original solution could be replaced with pesticide solution (Figure 12).

For initial testing, the bottle was filled with water and weighed before and after each spray. This gave promising results; after each spray the bottle weighed 95 ± 5 mg less, showing that around 100µL of water was being sprayed consistently.

The bottle was then filled with a solution of atrazine and mounted above a disc. The spray mechanism was operated once and the disc then assayed for atrazine by High Performance Liquid Chromatography (HPLC). The amount of atrazine applied had a range of ±70%. Various heights and angles were tested but none produced acceptably reproducible results. This may have been because, while the spray released the same amount of liquid each time, the plume dimensions were not constant. As a result, because the disc only represented a small portion of the spray area, small changes in droplet density or size had a large effect.

Figure 13 - 0.25 µL pesticide droplets on 1 cm² PET

Another approach was to apply the pesticide to the platform as separate droplets. A micropipette, which could dispense volumes down to 0.1 µL, was used. Droplets of 0.25 µL were applied to the platform, representing a coarse spray (Figure 13). When 16 x 0.25 µL droplets of atrazine at a concentration of 20 mg/ml were applied, a mean deposition of 81 ± 3.2 µg was found with a range of less than 10% (n=10). This showed that the application method was acceptably reproducible, however, using this technique, the maximum number of droplets that fit within 1cm² was found to be 20. Therefore, to achieve a loading of 80 µg/cm² would require the concentration of the solution to be 16 mg/ml, which is simply not realistic for a spray dilution. When 80 µL

of a 1 mg/ml atrazine solution was applied as a single droplet, the recovery upon extraction of platforms was 80.2 ± 1.2 μg with a range of less than 5%.

2.1.3 Transfer

As described above, the Belsey method involved a pesticide-coated disc being pressed against the skin for the duration of the diffusion experiment. This is not representative of the scenario in the field, where only intermittent contact between skin and foliage would occur and the residue would be transferred to the skin, for example by brushing against the contaminated surface. A method to transfer residue to the skin, based around this scenario, was therefore developed.

Three platform materials were tested for transfer efficiency; steel, glass and aluminiumised PET. Twenty 0.25 μL droplets of a 16 mg/ml atrazine solution were applied to the disc using the droplet application method described above and a glass vial was attached to the back of each disc in order to allow easy manipulation. The disc was then placed on to a piece of filter paper (which acted here as a substitute for the skin to test the methodology) and then rotated three times clockwise and three times anticlockwise, to transfer the residue (Figure 14). Atrazine was subsequently extracted and quantified from the filter paper and the disc, and the results are summarised in Table 1 below.

Figure 14 - A disc attached to the bottom of a glass vial to measure transfer of pesticide to filter paper

The percentage transfer to the filter paper was least from the aluminiumised PET and greatest from the glass disc. The AFM disc had the most reproducible transfer (standard deviation of 5.89% and highest and lowest values of 85.5% and 69.25% respectively). Importantly, these results also indicated that it is possible to estimate the amount transferred, from the difference between the quantity originally on the disc and that remaining post-transfer.

	Atrazine (% of dose applied):		
Platform	Filter Paper	Disc	Total
Glass	90.4±18.1	14.5±5.3	105.0±15.7
AFM	75.8±5.9	21.5±4.5	97.3±4.1
Aluminiumised PET	63.5±11.9	37.4±10.3	100.9±4.4

Table 1 - Percentage of dose applied transferred to filter paper n=10

To investigate how well the residue was spread on the skin, a dye (Nile Red) was dissolved in a blank emulsifiable concentrate formulation (supplied by Syngenta) and diluted 100x in water to create a relevant spray dilution. With this model AI it was possible to visualise the distribution of the residue using this transfer technique. Nile Red was considered a suitable model compound

as its physicochemical properties are similar to some of the pesticides considered later in the thesis. 40 µl of the solution was applied to a steel disc in a single droplet and allowed to dry. The rotation transfer procedure described above was performed on pig skin (n=5) and images taken of the resulting distribution of Nile Red.

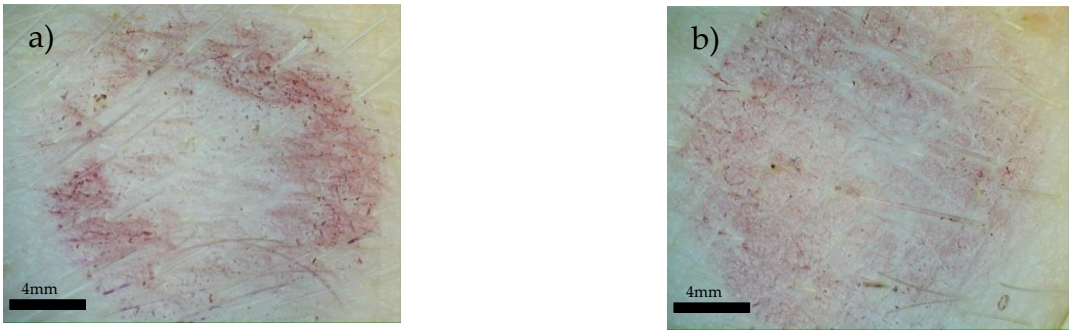

Figure 15 - Nile Red residue transferred to pig skin using (a) rotation method (b) rotation method with further lateral movements.

Upon inspection, it was evident that the residue was predominantly being applied to the periphery of the skin, leaving a blank area in the middle (Figure 15(a)). This is because the residue has dried on the disc in a "coffee ring" shape. Also, if rotated around the centre axis, residue at the edge of the disc will move a considerably longer distance than that at the centre. This greater amount of movement at the periphery may results in more transfer. To address this, as the diameter of the disc and vial (12mm) was slightly smaller than the confines of the diffusion cell lid (16mm) it was possible to also move the disc laterally upon the skin surface. This alternative transfer method was adopted, whereby, in addition to being rotated, the disc was also moved in a 'plus (+)' movement upon the skin and later in the thesis, in a circular motion also. The exact movements are not necessarily important but the idea was to

vary the movement of the disc to achieve an even spread of residue across the skin. Figure 15(b) shows the skin after application of Nile Red using this improved transfer method. The AI coverage is much more even and is spread across almost the whole area.

The optimised method also showed better reproducibility in the amount transferred and it was again possible to estimate the amount applied by measuring the quantity remaining on the disc (Table 2).

In this case, 20 μL of an emulsified concentrate formulation of trinexapac-ethyl (TXP, Syngenta) (10% w/w), diluted 100 times in water (to 1 mg/ml) was pipetted onto steel AFM discs and allowed to dry to form a residue. The transfer protocol was then carried out as described above. Immediately after application, three tape strips were taken in order to investigate the disposition of the residue on the skin. Pesticide was extracted from the tapes and the rest of the skin and quantified by HPLC.

Disc Remainder	Tape 1	Tape 2	Tape 3	Skin	Transferred	Total
2.2±0.3	11.9±1.0	2.8±0.5	0.8±0.2	2.2±0.7	17.7±1.0	19.8±0.8

Table 2 - Rotating disc & plus formation results on pig skin, all values in μg. n=5

The mean amount applied to the skin was the total amount of TXP recovered from the three tapes and the remaining skin: 17.7 ± 1.0 μg. This shows very good reproducibility with a narrow standard deviation. It is interesting that even after three tape strips have been taken 11% of the applied dose still remained within the skin. This suggests that some residue is deposited in crevasses and/or appendages of the skin.

2.2 Final Methods

With the issues associated with using leaves described above, the benefits of using an inert surface were deemed to outweigh the fact that it is less realistic. An inert surface is easier to handle in the lab, removes the need to source fresh leaves, and provides more reproducible residue deposition. Based on results from the transfer tests, 12 mm steel AFM discs were chosen for use in the final method. If deemed necessary, it should be possible to adapt the method for use with leaves, or a more representative platform, if available in the future.

Although the use of droplets may be the most realistic method, limitations in the amount that can be applied and a less reproducible application meant that this approach was not pursued. Application of pesticide solution as one droplet to the surface of the platform was the method carried forward. It is thought that the residue produced will be similar to that from smaller droplets. As it is to be transferred to the skin anyway, any effect on the final outcome should be minimal. It may also be possible to adapt the method to use a track sprayer to coat the discs in the future.

The final transfer method consists of three rotations clockwise, three rotations anticlockwise, and three movements in a '+' formation. For later experiments using other compounds, three movements in a circular motion were added to improve spreading.

2.2.1 Protocol

The final method used was based on the standard approach for measuring pesticide dermal absorption when seeking regulatory approval as advised by EFSA[8] and OECD[90] guidance.

General aspects of the methodology are described here; specifics and any protocol deviations are discussed in the relevant later chapters of the thesis.

Static Franz diffusion cells were used with a receptor chamber of 7.4 ml and an area of exposed skin of 2 cm^2. The receptor chamber was constantly mixed using a magnetic stirrer. As opposed to using a water jacket system, diffusion cells were incubated at 32 ± 1^0C in a controlled environment cabinet. Relative humidity was monitored but not controlled, the highest recorded % RH value for any experiment was 45% and the lowest was 39%. This approach reduced variations in temperature and humidity at the surface of the skin that would otherwise depend upon time of day and year. This was deemed important, as dissolution at the skin surface may be rate limiting for residue absorption.

Pig skin was obtained from a local abattoir, the hair trimmed with scissors, dermatomed to a nominal thickness of 750 μm (Zimmer®, Ohio, US) and subsequently stored at -20°C within 24 hours of slaughter. Over the course of the work, skin from 5 pigs was used; however, for all replicates within each individual study, skin from the same pig was used.

All receptor solutions contained phosphate buffered saline (PBS) pH 7.4, to which, for some compounds, Volpo™ was added to increase solubility. Solubility of each test compounds in the receptor solution was experimentally determined (given in Table 3) and were at least 10 times great than the highest observed receptor solution concentration.

Skin was allowed to thaw for a minimum of 30 minutes. Skin was then clamped between the two chambers, receptor solution added, and allowed to equilibrate at room temperature for a minimum of 20 minutes before application of pesticide.

Application of solution to the skin was by positive displacement pipette, the dose was spread across the entire skin surface. For each set of cells, a 'mock dose' was pipetted in to a vial and the quantity of AI was determined.

2.2.1.1 Residue Application

For residue application, pesticide solution was applied to 12 mm AFM discs and allowed to dry for ~24 hours at room temperature. Extra 'mock discs' were also made; these discs were extracted and quantified for AI after the drying period to check AI loading levels. Discs were attached to a weighted vial (~10 g) by double sided adhesive tape (Figure 16). Each disc was placed residue side down against the skin and rotated three times clockwise, three times anticlockwise, three times in a plus formation (Chapter 3) and three times in a circular motion (Chapters 4 and 5). The disc was removed from the skin and placed in a vial with 4ml of extraction solution to quantify the amount of AI remaining. This remaining amount was then subtracted from the original amount applied, giving an estimate of how much was transferred to the skin for each cell.

Figure 16 - Application of a dried pesticide residue to skin mounted in a Franz diffusion cell

After application, cells were immediately placed into the controlled environment cabinet and the protocol for liquid and residue applications was identical from this point on.

Prior to sampling, the receptor solution was thoroughly mixed by removal and replacement of 0.5 mL of solution three times. The sample was then taken by removing 0.3 mL for analysis which was immediately replaced with fresh receptor solution. Samples were taken at 2,3,4,5,6,7,8 and 24 hours for TXP and only 8 and 24 hours for PPZ, CLF and DFZ. This is because levels in the receptor were below or close to the LOQ for these three compounds.

A wash procedure was carried out 8 hours after initial exposure. This consisted of applying 100 μL of a 0.1% w/v soap solution onto the surface of the skin followed by immediate swabbing with two cotton Q-tips. Swabs were extracted in 1 mL (Chapter 3) or 4 mL (Chapters 4 and 5) of the relevant extraction solution.

The experiment was terminated at 24 hours from initial exposure, a final receptor solution sample was taken and tape stripping was performed. For each cell 15 adhesive tapes (scotch book tape, 3M, Germany) were cut to size 2 cm by 2 cm. For experiments in Chapter 3 and experiments investigating the effect of AI in Chapter 4, tapes were weighed before and after tape stripping on a balance (precision of 0.1 μg). As data obtained from weighing tapes was not deemed to add value to the results, tapes were not weighed for later experiments that investigated washing, loading and formulation.

Prior to tape stripping, parts of the skin outside of the exposure area were cut away. The skin was pinned down with a 2 cm^2 circular template around the exposure area. Each tape was pressed against the surface of the skin and removed in sequence. Each tape was extracted separately in 1 mL of extraction

solution. Following the tape stripping procedure, the remaining tissue was placed in an extraction vial and remaining AI extracted with 4 mL extraction solution. The lid from the donor compartment was extracted in 5 mL of extraction solution. All extracted samples were quantified by HPLC analysis (Table 3). Mean extraction efficiencies were shown to be greater than 95% from the disc, tape strips, wash swabs and remaining tissue.

Over the course of the work, four compounds Trinexapac-ethyl (TXP), clodinafop-propargyl (CLF), propiconazole (PPZ) and difenoconazole (DFZ) were used. A Shimadzu LC-20101A HPLC was used for analysis throughout the study, with a 25 cm C18 column HiQ sil C18HS (Particle Size: 5 μm , Pore Size : 100 Å). The injection volume was 50 μL for all samples. All samples were filtered prior to injection, Nylon or reconstituted cellulose (Cronus, nylon 0.45 μm, Labhut, UK and Minisart RC4, 0.45 μm, Fisher, UK).

Table 3 - HPLC methods used for quantification of all samples.

Compound	Mobile phase	Oven temp. (°C)	UV detection wavelength (nm)	Flow rate (ml/min)	Retention time (min)	Extraction solution†	Limit of Quantitation (LOQ) (μg/ml)	Solubility, 6% volpo in PBS (mg/ml)‡
CLF	70/30 ACN:H_2O	25	226	1.0	8.6	70/30 ACN:H_2O	0.035	0.5±0.2
CLF *	75/25 ACN:H_2O	25	226	1.0	6.7		0.035	[0.5% Volpo - 0.11±0.04]
DFZ	70/30 ACN:H_2O	35	212	1.5	6.9	80/20 ACN:H_2O	0.025	1.4±0.3
PPZ	70/30 ACN:H_2O	25	220	1.5	6.1	70/30 ACN:H_2O	0.07	2.0±0.2
TXP	60/40 ACN:0.1%H_2PO_4	25	280	1.0	8.5	60/40 ACN:H_2O	0.03	16.7±1.1 [PBS- 9.2±0.8]

*Method used specifically for CLF analysis of extracted stratum corneum tape strips.

† Extraction volumes were 1ml for tape strips, 4ml for remaining skin, swabs and discs and 5ml for the donor lid

‡ Experimentally determined, mean± SD (n=10)

3 PAPER 1 - *IN VITRO* METHOD TO QUANTIFY DERMAL ABSORPTION OF PESTICIDE RESIDUES.

Following the design of methodology to measure pesticide absorption from dried residues, as described above, the work in this paper aimed to test this approach.

Aims:

- To test the applicability and repeatability of the method designed to apply pesticide residues for *in vitro* diffusion cell studies
- To investigate if dermal absorption of trinexapac-ethyl is different when applied as a residue compared to when applied as a spray dilution
- Investigate the possibility of a 'wash-in' effect for the residue

3.1.1 Contributions

This Rapid Report was published in 'Chemical Research in Toxicology' in February 2015, all practical work described was carried out by the first author. The manuscript was written by the first author and edited and approved by all authors.

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Chemical Research in Toxicology

Rapid Report

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In Vitro Method to Quantify Dermal Absorption of Pesticide Residues

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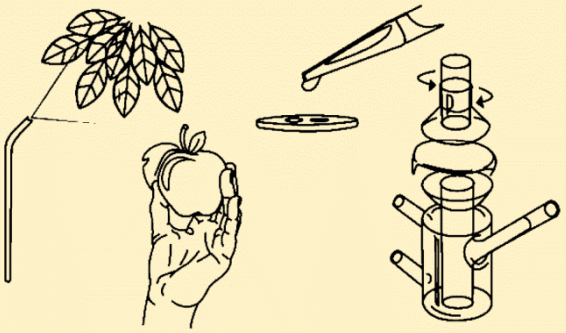
Supporting Information

ABSTRACT: All pesticides must go through a rigorous risk assessment process in order to show that they are safe for use. With respect to dermal risk assessment for re-entry workers, the absorption value applied to predict systemic dose from this external exposure is obtained by testing liquid forms of the pesticide *in vivo* and/or *in vitro*. However, in a real exposure scenario, the worker would be exposed to a dried residue, for which little or no absorption data are available. This study has developed a novel methodology for assessing the dermal absorption of pesticides from dried residues and aims ultimately to use this methodology to obtain more realistic absorption values for the risk assessment.



A principal function of the skin is to act as a barrier, both to the loss of endogenous water and to the absorption of exogenous compounds.[1] The skin comprises two major components: the innermost dermis and the superficial epidermis. Barrier function resides in the outer layer of the epidermis, the stratum corneum (SC), the thickness of which is typically on the order of 20 μm.

When a crop is treated with pesticide, a residue is left behind on surfaces such as leaves. An individual who subsequently enters the area may then be exposed to these residues via contact with his or her skin; this is most common with "re-entry workers" who may enter the treated area after application of the pesticide formulation to carry out tasks such as crop inspection or manual harvest.[2]

A risk assessment must be carried out for these re-entry workers and their potential dermal exposure (PDE, μg/day) calculated:[2]

$$\mathrm{PDE} = \mathrm{DFR} \times TC \times T$$

where DFR is the dislodgeable foliar residue (μg/cm^2), the quantity of substance remaining on the surface of the leaf that can be dislodged and transferred to skin; TC is the transfer coefficient (cm^2/h), which is specific to the particular re-entry task and refers only to the amount of contact between skin and the contaminated surface; and T (h/day) is the exposure time, typically 2 h for crop inspection and 8 h for harvest.

Once the potential exposure has been calculated, the percentage of the applied "dose" that becomes available systemically is estimated. *In vitro* skin absorption studies are carried out for most pesticides, determining the compound's uptake from a finite dose of both the concentrate and from a relevant in-use spray dilution (described below). The higher of the calculated percentage absorption values (generally the most dilute solution) is then used to represent a worst-case scenario for the re-entry worker. To pass risk assessment, this value must be below the maximum acceptable value identified for the compound during toxicology testing.[3]

In a real exposure scenario, the re-entry worker would most likely come into contact with a dried residue rather than a liquid form of the product. Unfortunately, no acceptable methodology exists for the acquisition of absorption data from such residues. It is likely that the use of data from liquid applications represents an overestimate and that the dried residues left on plant surfaces, to which workers are exposed, would not be absorbed to the same extent. This may lead to the pesticide failing the risk assessment process meaning that safe and effective products may not be approved for use. Previous work[4] has shown that pesticide absorption from a residue, when applied in the form of a coated disk pressed against the skin, was different from that of an aqueous solution. However, this occlusive and long-term exposure was not fully representative of a re-entry worker scenario, where only brief contact between skin and foliage would occur. Furthermore, the "doses" used (100−1000 μg/cm^2 of pesticide) were an order of magnitude higher than would occur in a re-entry exposure scenario and were delivered as neat active ingredients either in solution or as a suspension (as opposed to a commercially relevant formulation).

The aim of this study, therefore, is to develop a robust methodology for assessing the dermal absorption of pesticides from dried foliar residues that addresses these limitations and is more relevant to the re-entry scenario. The ultimate aim is to use this approach to obtain more realistic absorption values for

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risk assessment. It is important that this method is as close to a real exposure scenario as possible and is easily reproducible.

A standard *in vitro* protocol was used to measure pesticide dermal absorption.[5,6] Experiments (n = 4−5) were performed in static Franz diffusion cells (Permegear, Hellertown, U.S.A.), with dermatomed porcine skin (diffusion area = 2 cm^2) maintained at 32 °C. The receptor chamber contained 7.4 mL of a 6% (w/v) solution of polyoxyethylene glycol (10) oleyl ether (Sigma, U.K.) in phosphate-buffered saline at pH 7.4. The pesticide Trinexapac-ethyl (TXP, Syngenta plc, Jealott's Hill, U.K.) was applied to the skin as an emulsifiable concentrate (10% w/w) diluted 100-fold in water, or as a dried residue (see below).

In the case of the liquid formulation, 20 μL (1 μg/μL) was applied directly and evenly to the skin surface. For the residue, 40 μL of the diluted concentrate was first applied to a 12 mm diameter steel disc (SPM specimen discs, TAAB Laboratories Equipment Ltd., Aldermaston, U.K.) and allowed to dry for 24 h to a dried residue. The disc was then attached to a weighted vial (~10 g) that was rotated on the skin surface. The procedure involved three complete rotations in both the clockwise and anticlockwise directions, followed by moving the disc laterally in a "+" configuration (see Supporting Information). After application, nontransferred residue remaining on the disk was extracted and quantified to confirm the amount actually transferred to skin, specifically 21.7 ± 3.3 μg, (mean ± S.D., i.e., ~54% of that applied to the disk) with the aim being to match the 20 μg application from the liquid. Postapplication of the formulations, the receptor solution was sampled at 2, 4, 6, and 8 h. The skin surface was washed at 8 h (to represent a typical working day) with 100 μL of a mild (0.1% w/v) soap solution and dried with two cotton buds. An additional receptor solution sample was taken at 24 h, after which the stratum corneum was sequentially removed by adhesive tape stripping.[4,7] The first two tape-strips were not discarded, and the chemical thereon was quantified; however, the quantities found were not included in the total absorption calculations as this material is generally not assumed to be bioavailable.[3]

The skin uptake and absorption of TXP (Table 1; Figure 1) was determined following HPLC analysis (see Supporting Information) of the receptor solution samples, the SC tape-strips, the washing solution, viable tissue, and cotton buds. The pesticide was efficiently extracted from the SC using 60:40 acetonitrile/water.

Table 1. Skin Uptake of TXP (Mean ± SD)[a]

	liquid (μg)	residue (μg)	p-value
receptor 2 h	0.08 ± 0.17	0.00 ± 0.00	0.35
receptor 4 h	0.52 ± 0.18	0.00 ± 0.00	<0.01
receptor 6 h	0.72 ± 0.12	0.00 ± 0.00	<0.01
receptor 8 h	0.90 ± 0.16	0.07 ± 0.15	<0.01
receptor 24 h	1.70 ± 0.46	1.15 ± 0.40	0.08
tapes 1 and 2	0.86 ± 0.45	0.56 ± 0.16	0.19
tapes 3−15	0.33 ± 0.11	0.23 ± 0.12	0.29
surface wash	11.0 ± 1.78	12.2 ± 2.67	0.42
skin	1.08 ± 0.66	0.30 ± 0.10	0.03
total absorbed	3.11 ± 0.86	1.68 ± 0.56	0.015
% "dose" absorbed	15.54 ± 4.36	7.63 ± 1.65[b]	<0.01

[a]Surface cleaned at 8 h. [b]Expressed as a percentage of the estimated "dose" applied for each replicate.

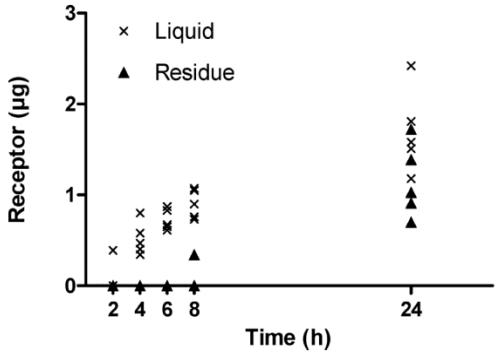

Figure 1. Permeation of TXP into the receptor (μg) as a function of time. Skin surface cleaned at 8 h.

Total TXP absorption (i.e., quantity of pesticide in tape strips 3−15 + skin extraction + quantity permeated to receptor) was significantly lower for the residue than for the liquid; permeation of pesticide into the receptor solution continued after the skin was washed. It is noteworthy that, while significantly more TXP permeated into the receptor phase in 8 h following liquid application, there was no significant difference after 24 h between the liquid and dried residue exposures.

It was unclear why pesticide absorption from the residue began slowly but then appeared to "catch up" with that from the liquid between 8 and 24 h. To investigate the possibility that the washing procedure somehow aided pesticide absorption from the residue, further experiments were conducted with the wash procedure carried out at 24 h (i.e., at the termination of the entire experiment) instead of at 8 h.[8]

Table 2 compares the results from the liquid and residue applications for the 24 h surface wash. At 4 and 6 h, as before,

Table 2. Skin Uptake of TXP (Mean ± SD)[a]

	liquid (μg)	residue (μg)	p-value
receptor 2 h	0.12 ± 0.17	0.00 ± 0.00	0.20
receptor 4 h	0.55 ± 0.19	0.00 ± 0.00	<0.01
receptor 6 h	0.80 ± 0.30	0.06 ± 0.11	<0.01
receptor 8 h	0.91 ± 0.33	0.51 ± 0.17	0.06
receptor 24 h	2.21 ± 0.61	1.95 ± 0.46	0.51
tapes 1 and 2	0.68 ± 0.16	0.76 ± 0.19	0.54
tapes 3−15	0.36 ± 0.10	0.36 ± 0.10	0.74
surface wash	7.22 ± 2.14	8.62 ± 1.19	0.28
skin	0.89 ± 0.12	0.77 ± 0.18	0.24
total absorbed	3.45 ± 0.57	3.10 ± 0.70	0.41
% "dose" absorbed	17.27 ± 2.87	15.05 ± 2.24[b]	0.22

[a]Surface cleaned at 24 h. [b]Expressed as a percentage of the estimated "dose" applied for each replicate.

more pesticide had penetrated to the receptor phase from the liquid application. Notably, at 8 and 24 h, there was no significant difference between the amounts of TXP that had reached the receptor from the liquid and residue applications. At the 8 h time point, the protocol in this experiment is identical to that described above, and all of the 8 h receptor solution quantities, from the two experiments, were therefore analyzed together. The amount that had penetrated the skin from the liquid was found to be significantly higher (p < 0.001).

For the residue, total absorption was significantly higher when washing was performed at 24 h instead of 8 h. Therefore,

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there is no evidence of a possible "washing-in" effect. Intriguingly, at 24 h, there was no significant difference between total absorption from the liquid and that from the residue. This may be due to (some) TXP residue dissolving into skin surface moisture (TXP is relatively water-soluble, 10 mg/mL). Alternatively, when applied as a solution, evaporation may transform the vehicle into a residue. As a result, as time progresses, the uptake/penetration of TXP from the dilute formulation and the dried residue are similar.

In summary, a novel, *in vitro* method has been developed with which to measure dermal exposure from dried pesticide residues under relevant "in-use" conditions. Further work is required to fully validate the approach for a range of typical pesticide formulations and for a range of "actives" of diverse physicochemical properties. It should also be emphasized that, for regulatory purposes, mass balance would be an essential requirement; for example, full washings of the diffusion cell would be required. In this regard, it is noted that the EFSA "guidance on dermal absorption"[3] recommends, when low chemical recovery is observed, that uptake data should be normalized and "expressed as a percentage of the total amount recovered" for each replicate. When this procedure is carried out with the data in Tables 1 and 2, and the normalized % absorption values statistically analyzed, the results point to exactly the same conclusions as those described above. Additionally, the impact of environmental moisture and concomitant skin hydration upon the degree of exposure would need to be assessed.

■ ASSOCIATED CONTENT

Supporting Information

HPLC method for TXP analysis and video demonstrating the method used to transfer the pesticide to the skin. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS

SC, stratum corneum; DFR, dislodgeable foliar residue; TC, transfer coefficient; *T*, exposure time; TXP, Trinexapac-ethyl; HPLC, high-performance liquid chromatography; EFSA, European Food Safety Authority

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Chem. Res. Toxicol. 2015, 28, 166−168

3.1.2 Further Discussion

This work showed that the method designed produces reproducible residue application. Applications were 21.7±3.2 μg in the 8-hour exposure experiment and 20.5±2.0 μg in the 24-hour experiment. The highest measured transfer was 26.4 μg and the lowest was 17.8 μg.

The results showed that TXP is absorbed significantly less from the residue than the spray dilution, when decontamination occurred at 8 hours. However, when the skin was not decontaminated until 24 hours, total absorption was not significantly different between the two application types. Between the two exposure times total TXP absorption from the liquid did not change, however, absorption from the residue was significantly greater after 24-hour exposure. This may be because absorption from the residue is limited by dissolution at the skin surface and therefore in the extra 16-hour exposure, TXP is still partitioning in to the skin, whereas absorption from the liquid is essentially complete by 8-hours.

Up to the 8-hour time point, the protocol for the two experiments was identical. However, for the residue experiments, when the amount of TXP in the receptor solution is plotted against time (Figure 17), penetration to the receptor is significantly higher between 6 and 8 hours for the 24-hour exposure experiment than for the 8-hour experiment and this appears to contribute significantly to the overall increased absorption.

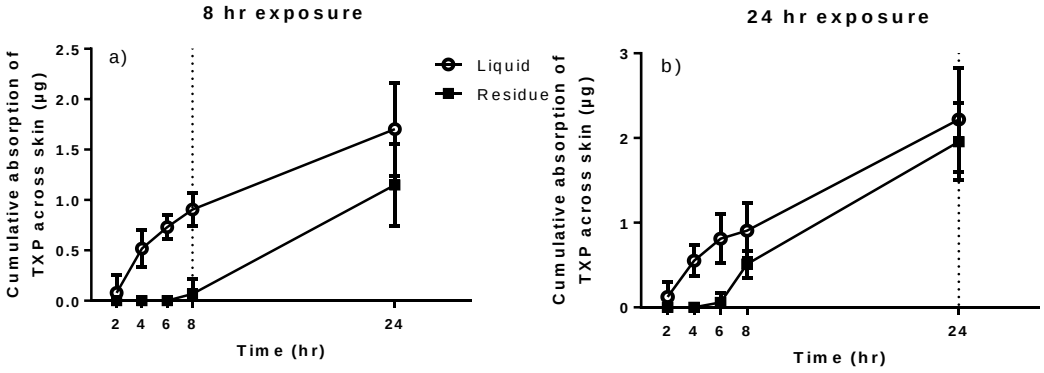

Figure 17 – Cumulative absorption of TXP across the skin in vitro (mean ± SD) following liquid spray dilution and residue applications as a function of time for a) 8-hour exposure (n= 5) b) 24-hour exposure (n= 4-5)

This apparent difference may be a consequence of the low concentration of these samples. Samples below LOQ (0.03 µg/ml for TXP) were reported as zero. One replicate in the 8-hour exposure experiment had an 8-hour receptor concentration of just above this level at 0.046 µg/ml (0.34 µg total in receptor), with the other 4 replicates containing '0 µg' however, with a receptor volume of 7.4ml they could potentially each contain up to 0.22 µg of TXP before reaching the LOQ. The receptor samples taken at 8 hours from the 24-hour exposure experiments were only just above the LOQ. Therefore, it is likely that the mean concentration of TXP in the receptor at 8 hours was higher than the results suggest for the 8-hour exposure experiment.

To test that the results of this experiment were valid and to draw more firm conclusions about the effect of exposure time, this experiment was repeated as well as adding four further shorter exposure times. These results are described in Chapter 4.

The absorption of three additional compounds following 8-hour exposure was also investigated. Although the work in Paper 1 demonstrated the potential of the methodology, for it to be fully validated a wider range of pesticides must be tested. Also, to draw more firm conclusions about absorption from residues in

general it is necessary to test compounds with varying physicochemical properties. TXP is relatively hydrophilic and therefore the stratum corneum is the primary barrier to its systemic absorption. Compounds that are more lipophilic and therefore dissolve more easily into the SC, could behave differently when presented as a residue.

4 PAPER 2 - DERMAL UPTAKE AND ABSORPTION OF PESTICIDE RESIDUES

Following validation of the methodology in Paper 1, the following study aimed to extend these findings by investigating absorption from three additional active ingredients in the same formulation. Absorption of one compound, TXP, was also measured following various exposure times, to investigate how a shorter exposure may affect dermal absorption from both liquid and residue applications.

4.1 Aims:

- To investigate the absorption of AIs with different physicochemical properties.
- To investigate the effect of different exposure times on dermal absorption of both spray dilution and dried residue.

4.2 Contributions

This manuscript was submitted to 'Environmental Science and Technology' in August 2017, all practical work described was carried out by the first author. The manuscript was written by the first author and edited and approved by all authors.

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4.3 Abstract

Current guidance for dermal exposure assessment of plant protection products typically uses *in vitro* skin penetration data for the active ingredient when applied as both the concentrated product and relevant spray dilutions thereof. However, typical "re-entry" scenarios involve potential skin exposure to a 'dried residue' of the spray dilution, from which the absorption of a pesticide may be quite different. The research reported in this paper has shown: [1] A method to transfer dried pesticide residues from an inert platform to the skin surface has been successfully developed that is reproducible for four active ingredients, of diverse physicochemical properties, after their application in a commercially relevant formulation. [2] The skin uptake and absorption of all four pesticides examined was significantly less from a dried residue than from a spray dilution; the difference, in general, was on the order of a factor of 2. [3] Decontamination experiments with one of the active ingredients tested (trinexapac-ethyl) showed that, post-exposure to a spray dilution, skin surface cleaning must be performed within 1 hour to significantly reduce potential systemic exposure (as assessed relative to continual contact for 24 hours); in contrast, after contact with a dried residue, the sooner decontamination was performed, the greater the decrease in exposure achieved, even when the time of contact was as long as 8 hours.

4.4 Introduction

When pesticides are used in practice, the application is usually in the form of a 'spray dilution' in which the concentrated formulation has been mixed with water. After application, the diluted formulation eventually leaves a 'dried residue' on surfaces, such as leaves or fruit. For each pesticide product, a series of risk assessments must be carried out before use. These calculations encompass various scenarios and consider the product's effect on the ecosystem and on a number of specific organisms, including humans. For example, an operator could potentially be exposed during mixing and loading of the product into a spray tank or, during application, there is a further possibility of exposure, not only to the operator, but also to bystanders and nearby residents; in addition, post-application, anyone entering the treated area may be at risk of exposure to the recently applied product.

A re-entry worker, an individual who enters a field to carry out a task such as crop inspection or harvest, may enter a treated area soon after pesticide application and risk exposure, therefore, to the dried residue remaining on leaves, fruit, etc. Exposure occurs most typically via dermal (the most important[7]) and inhalation routes, with secondary exposure also possible via hand-to-mouth transfer. The potential dermal exposure (PDE) can be estimated for the purpose of a risk assessment and depends upon: (a) how much pesticide is present on the contaminated surface, the so-called dislodgeable foliar residue (DFR), which is dictated by factors such as application rate, concentration of the active ingredient (AI), and the pesticide formulation; (b) how much of the DFR is subsequently transferred to the skin, as characterised by a transfer coefficient (TC), which is dictated by intensity of contact a worker has with the contaminated surface; and (c) the duration of the work. Once the PDE has been determined, the quantity of pesticide which will be absorbed through the skin and eventually become systemically available can be estimated.

The skin's primary function is to act as a barrier both to the loss of endogenous water and to the absorption of exogenous xenobiotics. The outermost layer, the non-viable stratum corneum (SC), is typically the rate-limiting barrier to absorption, meaning that *ex-vivo* skin has a competent SC[88] and can be used *in vitro* as a surrogate for the *in vivo* situation[89]. Consequently, at present, to determine the skin absorption of a pesticide, *in vitro* diffusion cell experiments on the concentrated product, and on representative spray dilutions, are performed. The highest fraction of the 'dose' absorbed from the three is then used for risk assessment, and this value is multiplied by the PDE to yield an estimate of systemic exposure.

However, as articulated above, in an actual *re-entry* scenario, a worker does not come into contact with the concentrate or a spray dilution of the pesticide; rather, skin contact occurs with the dried residue of the spray dilution. In previous experiments, a significant difference was observed in the dermal absorption of various pesticides from liquid and residue forms[21]; in some cases, the chemical was absorbed more from the residue but, in others, the uptake was less. The *in vitro* method used to measure skin uptake from the residue involved pesticide application to an artificial material, the coated surface of which was subsequently pressed against the skin for 8 hours (signifying a typical working day). An obvious limitation of this approach is that the skin is occluded by the disc throughout the exposure, and the resulting increased hydration has been shown to amplify dermal absorption[56, 57, 127]. This effect may be exacerbated for a dried residue, as surface moisture resulting from occlusion effectively becomes the 'vehicle' in this exposure. Another shortcoming of the protocol used was that the active ingredient was deposited on the artificial surface from a simple solvent rather than from a commercially relevant formulation. Furthermore, the lowest dose used was at the upper end of a realistic worker exposure. As many studies have shown percentage absorption often decreases with increasing skin

loading[65-67, 128], the use of doses higher than that to which a worker may be exposed may provide unrealistic absorption values.

To overcome these limitations, a method described recently[129] was used to measure the dermal absorption of pesticide residues in a realistic, non-occlusive manner. In the research described in the present paper, this refined approach was used to compare the dermal absorption of four pesticides from liquid and residue 'vehicles' following application from the same commercially relevant emulsifiable concentrate formulation. Furthermore, for one of the chemicals, the effect of decontamination after different exposure periods was investigated to better simulate, for example, hand-washing events prior to rest or lunch breaks in a worker's typical 8-hour day. Clearly, hand-washing can potentially remove a significant fraction of a dried residue and therefore reduce the overall systemic exposure[2]. Although it has been found that hand-washing does not completely decontaminate the skin[119,118], perhaps because mobilisation of material trapped in skin crevices and/or appendages is difficult and less than 100% efficient, the extent to which similar behaviour occurs with dried residues is presently unknown.

4.5 Methods

4.5.1 Materials

Four active ingredients (AI), spanning a range of physicochemical properties, were considered: trinexapac-ethyl (TXP), clodinafop-propargyl (CLF), difenoconazole (DFZ) and propiconazole (PPZ) (Table 4). Each was formulated in an identical commercially relevant, naphtha-based 10% w/v emulsifiable concentrate (EC) supplied by Syngenta (Jealotts Hill, U.K.). Spray dilutions of each AI were prepared by diluting the concentrate 100-fold in water, producing thereby a final AI concentration of 1 mg/mL.

Table 4 - Pesticides selected for investigation and their relevant physicochemical properties.

Compound	**MW**	**Melting point (°C)**	**Log P**	**Aqueous solubility (mg/cm^3)**	**Predicted J_{max} ($\mu g/cm^2/h$)[63, 64]**
Trinexapac-ethyl (TXP)	252	36.3	-0.29	10.2	0.366
Clodinafop-propargyl (CLF)	350	59.5	3.9	0.004	0.032
Difenoconazole (DFZ)	406	82.5	4.36	0.015	0.117
Propiconazole (PPZ)	342	-23	3.72	0.15	1.02

4.5.2 Dermal absorption procedures

In vitro skin uptake and absorption experiments were performed using static Franz diffusion cells with a receptor volume of 7.4 mL and area of 2 cm^2. Dorsal porcine skin from a single donor was dermatomed to a nominal thickness of 750 μm, frozen within 24 hours of slaughter and thawed before use. Porcine skin was chosen as it is considered to be the closest surrogate to human skin[96, 97, 130]. However, the structure of and penetration across pig skin does differ from that of human skin, and cross-species conclusions should be drawn with caution.

Using skin from the same donor for each set of experiments reduced variability in the results and facilitated comparison between different AIs and exposure periods. The method used followed OECD[90] and EFSA[8, 9] guidance documents for *in vitro* diffusion cell studies required in the regulatory approval process. The only significant deviation was that, instead of a water jacket system to control skin temperature, diffusion cells were incubated at 32 ± 1^{0}C, and at a relative humidity of 40 ± 5% in a controlled environment cabinet. This approach reduced variations in temperature and humidity at the surface of the skin that otherwise depend upon time of day and year. The tighter control of the environmental conditions at the surface of the skin was considered important for this study because of the potentially rate-limiting nature of the dissolution kinetics of the AI residue.

As a control, skin uptake and absorption of the liquid spray dilution of each AI was also assessed. A volume of 25-30 μL* was applied for a period of 8 hours (a typical working day), when the skin surface was cleaned.

The wash procedure involved application to the skin of 100 μL of a 0.1% w/v soap solution and cleaning with two cotton swabs, from which the AI was subsequently extracted and quantified. The receptor solution (which consisted of 6% Volpo™ (Sigma Aldrich Co., Gillingham, UK) in phosphate-buffered saline) was sampled at each hour from 2 to 8 hours for TXP and then at 24 hours. For the other three AIs, receptor solution samples were taken at 8 and 24 hours only.

** The exact volume used was informed by the average amount of AI residue transferred to the skin from the stainless-steel disc to which 40 μg had been applied (see following text) in preliminary experiments. The actual volumes in the control experiments were 25 μL for TXP and PPZ, and 30 μL for DFZ and CLF.*

After the final receptor phase sampling at 24 hours, the skin was removed from the diffusion cell and the outer, stratum corneum was removed by adhesive tape-stripping (Scotch Book Tape, 3M, Germany) as previously described[21]. According to the EFSA guidelines[8], AI in the first two tape strips is considered to be non-absorbed material lost *via* desquamation, while that in the subsequently removed 13 tape strips is assumed to be absorbed, as is the pesticide recovered from the remaining skin tissue post-stripping.

The methods used to ensure efficient extraction of the AIs from the stratum corneum tape-strips and from the remaining skin post-stripping are described in the supplementary information, as are the HPLC analytical protocols for each AI. 'Total absorption' of the AI was therefore determined as the sum of the cumulative amount of pesticide in (a) stratum corneum tape-strips 3-15, (b) the skin remaining post-stripping, and (c) the receptor solution at 24 hours. AI in the first two stratum corneum tape-strips was also extracted and quantified but not included in the total absorption reported.

To measure AI absorption from a 'dried' residue, exactly 40 μL of the liquid spray dilution was applied to a stainless-steel disc of 12 mm diameter (SPM specimen discs, TAAB Laboratories Equipment Ltd., Aldermaston, U.K.) and allowed to dry for 24 hours. This residue was then applied to the skin with a standardised transfer procedure[129]. In addition to the circular rotations and lateral movements described previously, the disc was also moved three times in a circular motion in order to ensure adequate spreading. AI remaining on the disc post-transfer was then extracted and quantified allowing an estimate of the AI residue transferred to the skin to be determined by difference. After application of the residue to the skin, the *in vitro* diffusion cell experimental protocol was identical to that for the liquid application explained above.

For TXP, additional skin uptake and absorption experiments were performed to assess the impact of skin washing after different exposure periods. Identical procedures to those already described were used with four modifications: (i) the skin surface was cleaned after separate exposure durations of 0.5, 1, 2, 4, 8 and 24 hr; (ii) the receptor solution was phosphate-buffered saline alone, (iii) the skin used was from a different (but single) pig; and (iv) the receptor solution was also sampled after 1 hour.

It is important to note that the aim of this study was not to obtain absorption factors for regulatory assessment, but rather to further validate the methodology for residue application and to assess differences in absorption from liquid and residue applications.

4.5.3 Results & Discussion

The 24-hour skin uptake and absorption of the four AIs considered (in terms of the % of the applied 'dose') is summarised in Figure 18. Given the close overlap between the actual amounts of the AIs applied, the expression of the data as % of the applied quantity allows a valid comparison between the chemicals, and between uptake and absorption from liquid and residue phases, to be made. For all four AIs, the total % of the applied amount was significantly higher ($p < 0.05$) from the liquid than from the residue. Across the different AIs, for both liquid and residue applications, there was about a 2 to 3 fold range in the absolute skin uptake and absorption values.

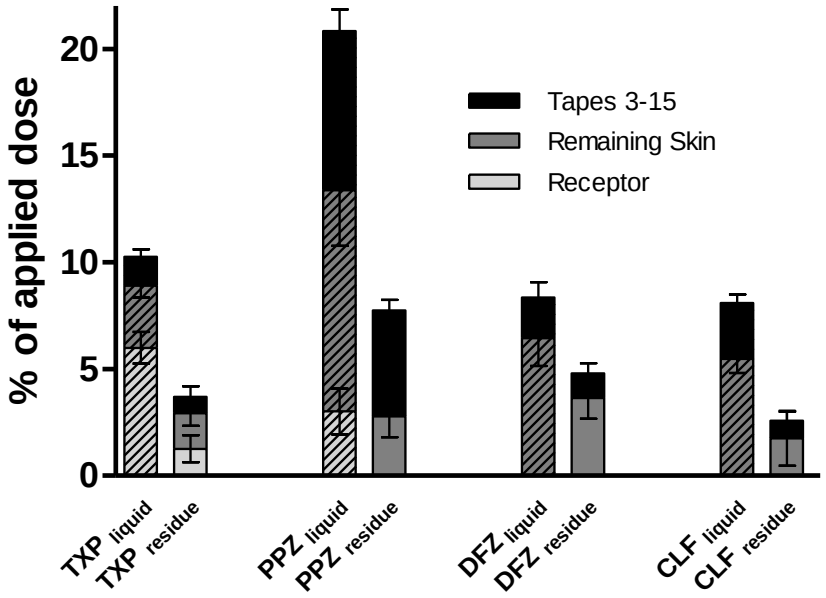

Figure 18 - Stacked bar chart showing the skin uptake and absorption at 24 hours after liquid (hatched bars) and residue (plain bars) application of the pesticides considered (mean ± SD; n = 3-11).

More detailed skin uptake and absorption data for TXP are shown in Table 5. From the liquid spray dilution application, TXP penetration through the skin to the receptor phase was detectable from the first measurement at 2 hours and increased progressively over the duration of the experiment (Figure 19 (a)). The derived rate of penetration, however, indicated a clear maximum at around 4 hours (Figure 19 (b)). Given that only ~10% of the applied TXP was absorbed over the entire 24 hours of measurement, the peak in the absorption rate cannot be attributed to depletion of the 'dose' applied. Rather, it is more likely that the downturn in the rate of penetration is the result of the liquid spray dilution drying out and leaving a solid residue after a certain time from which absorption is much slower; that is, the pesticide now needs to re-dissolve in the limited surface moisture available before it can diffuse into the skin.

Table 5- Skin uptake and absorption results for TXP (mean ± SD; n = 3 for the liquid application, n = 7 for the residue).

Application (washed at 8 hours)	**Liquid**	**Residue**
TXP applied (µg)	25	22.45 ± 4.25
TXP recovered in swabs (µg)	11.12 ± 1.63	11.06 ± 2.38
TXP disposition		
SC tape-strips 1-2 (µg)	1.20 ± 0.28	0.53 ± 0.24*
SC tape-strips 3-15 (µg)	0.33 ± 0.09	0.17 ± 0.12
SC tape-strips 1-15 (µg)	1.53 ± 0.37	0.70 ± 0.33*
Remaining skin (µg)	0.73 ± 0.14	0.38 ± 0.16*
Receptor phase at 2 hr (µg)	0.32 ± 0.13	< LOQ
Receptor phase at 4 hr (µg)	0.76 ± 0.22	< LOQ
Receptor phase at 6 hr (µg)	0.96 ± 0.20	< LOQ
Receptor phase at 8 hr (µg)	1.12 ± 0.23	< LOQ
Receptor phase at 24 hr (µg)	1.50 ± 0.18	0.27 ± 0.15*
Total TXP uptake/absorption (µg)#	2.56 ± 0.18	0.82 ± 0.34*
% uptake/absorption of TXP applied	10.25 ± 0.40	3.72 ± 1.39*

*Significantly smaller ($p < 0.01$) than the liquid application value (Student's unpaired t-test).

< LOQ = below the limit of quantitation of TXP.

#Sum of (SC tape-strips 3-15) + (Remaining skin) + (Receptor phase at 24 hr).

The results from the residue application support this interpretation. In this case, TXP was only detected in the receptor at 24 hours, indicative of a much longer lag-time (and reflecting the slow dissolution step referred to above). This is consistent with a previous study[129] involving a similar protocol. This study involved an additional set of experiments where the skin was not washed until 24 hours post-application, here an early peak rate of absorption was again seen for the liquid application but, over the 8-24 hour period, the fluxes of TXP from the liquid and from the residue were essentially the same (0.08 and 0.09 µg/hr, respectively).

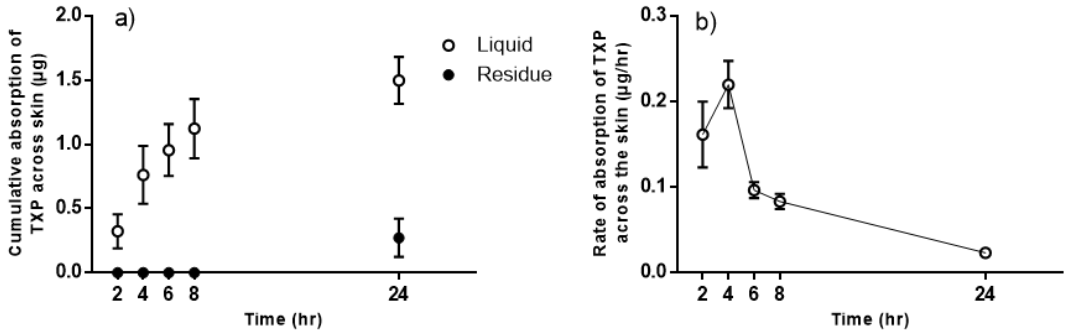

Figure 19 - (a) Cumulative absorption of TXP across the skin in vitro (mean ± SD) following liquid spray dilution (n = 3) and residue (n = 7) applications as a function of time. (b) Rate of absorption of TXP across the skin in vitro following application of a liquid spray dilution as a function of time (mean ± SD; n = 3).

In terms of the overall skin disposition of TXP, the total absorption (expressed either as an absolute quantity or as a % of the 'dose' applied) was 2.8 times larger from the liquid spray dilution, than from the residue; this difference was significant at $p < 0.01$. Differences in amounts recovered from the stratum corneum tape-strips and the rest of the skin were somewhat smaller (closer to 2-fold) but were again statistically significant.

Table 6 - Skin uptake and absorption results for PPZ (mean ± SD; n = 6 for both liquid and residue applications).

Application (washed at 8 hours)	**Liquid**	**Residue**
PPZ applied (µg)	25	24.44 ± 1.28
PPZ recovered in swabs (µg)	12.59 ± 3.30	19.95 ± 1.53§
PPZ disposition		
SC tape-strips 1-2 (µg)	2.83 ± 1.22	1.87 ± 0.40
SC tape-strips 3-15 (µg)	1.86 ± 0.25	1.33 ± 0.16*
SC tape-strips 1-15 (µg)	4.69 ± 1.31	3.20 ± 0.47†
Remaining skin (µg)	2.58 ± 0.65	0.75 ± 0.26*
Receptor phase at 8 hr (µg)	< LOQ	< LOQ
Receptor phase at 24 hr (µg)	0.75 ± 0.27	< LOQ
Total PPZ uptake/absorption (µg)#	5.19 ± 0.99	2.08 ± 0.37*
% uptake/absorption of PPZ applied	20.77 ± 3.96	8.50 ± 1.36*

§Significantly greater ($p < 0.01$) than the liquid application value (Student's unpaired t-test).
*Significantly smaller ($p < 0.01$) than the liquid application value (Student's unpaired t-test).
†Significantly smaller ($p < 0.05$) than the liquid application value (Student's unpaired t-test).
< LOQ = below the limit of quantitation of PPZ.
#Sum of (SC tape-strips 3-15) + (Remaining skin) + (Receptor phase at 24 hr).

As indicated in Table 6, PPZ was the most efficiently taken up of the four pesticides from both liquid and residue applications with liquid being absorbed 2.4 times more (20.8% and 8.5%, respectively, in terms of % 'dose' applied, a significant difference at $p < 0.01$). Penetration of the chemical to the receptor phase, however, was measurable for the liquid spray dilution application only at 24 hours; no samples reached LOQ at 8 hours. For the residue, PPZ in the receptor did not reach the LOQ at either 8 or 24 hours. The relatively high solubility of PPZ in the receptor solution (2 mg/mL) suggests that the compound's high lipophilicity is the likely reason behind this observation.

Uptake of PPZ into the skin – both in terms of the amounts in stratum corneum tape-strips 3-15 and in the remaining skin – was significantly higher ($p < 0.01$) for the liquid application. This was slightly surprising as the melting point of this chemical is -23°C, indicating that it is a liquid at ambient temperature and that, when left as a residue, therefore, a lesser resistance to dissolution might have been expected.

Table 7 - Skin uptake and absorption results for DFZ (mean ± SD; n = 6 for the liquid application, n = 11 for the residue).

Application (washed at 8 hours)	**Liquid**	**Residue**
DFZ applied (µg)	30	29.01 ± 3.44
DFZ recovered in swabs (µg)	24.31 ± 1.88	22.45 ± 3.32
DFZ disposition		
SC tape-strips 1-2 (µg)	1.55 ± 0.59	1.16 ± 0.33
SC tape-strips 3-15 (µg)	0.57 ± 0.21	0.34 ± 0.15[†]
SC tape-strips 1-15 (µg)	2.12 ± 0.64	1.49 ± 0.45[†]
Remaining skin (µg)	1.93 ± 0.39	1.05 ± 0.28[*]
Receptor phase at 24 hr (µg)	< LOQ	< LOQ
Total DFZ uptake/absorption (µg)[#]	2.50 ± 0.52	1.38 ± 0.35[*]
% uptake/absorption of DFZ applied	8.34 ± 1.74	4.79 ± 1.13[*]

[†]Significantly smaller ($p < 0.05$) than the liquid application value (Student's unpaired t-test).

[*]Significantly smaller ($p < 0.01$) than the liquid application value (Student's unpaired t-test).

< LOQ = below the limit of quantitation of DFZ.

[#]Sum of (SC tape-strips 3-15) + (Remaining skin) + (Receptor phase at 24 hr).

Table 8 - Skin uptake and absorption results for CLF (mean ± SD; n = 6 for both liquid and residue applications).

Application (washed at 8 hours)	**Liquid**	**Residue**
CLF applied (µg)	30	29.97 ± 2.64
CLF recovered in swabs (µg)	21.09 ± 2.56	23.74 ± 4.81
CLF disposition		
SC tape-strips 1-2 (µg)	2.24 ± 0.47	1.25 ± 0.52*
SC tape-strips 3-15 (µg)	0.79 ± 0.12	0.25 ± 0.14*
SC tape-strips 1-15 (µg)	3.03 ± 0.42	1.50 ± 0.65*
Remaining skin (µg)	1.64 ± 0.19	0.54 ± 0.41*
Receptor phase at 24 hr (µg)	< LOQ	< LOQ
Total CLF uptake/absorption (µg)#	2.43 ± 0.30	0.79 ± 0.47*
% uptake/absorption of CLF applied	8.09 ± 1.00	2.58 ± 1.42*

*Significantly smaller ($p < 0.01$) than the liquid application value (Student's unpaired t-test).

< LOQ = below the limit of quantitation of CLF.

#Sum of (SC tape-strips 3-15) + (Remaining skin) + (Receptor phase at 24 hr).

The results for DFZ and CLF are presented in Table 7 and Table 8, respectively, and are relatively similar. For both chemicals, skin uptake was significantly higher ($p < 0.01$) from the liquid application than from the residue by factors of approximately 1.8 (DFZ) and 3.1 (CLF). Permeation of the two pesticides, from both applications, was never detectable in the diffusion cell receptor phase, even after 24 hours. As for PPZ, this was not due to the limited solubilities of DFZ and CLF in the receptor phase where both compounds had more than adequate values (1.4 and 0.5 mg/mL, respectively).

TXP absorption from liquid and residue applications, when the skin was cleaned after different exposure periods is summarised in Figure 20. Tabulated results are in the Appendix.

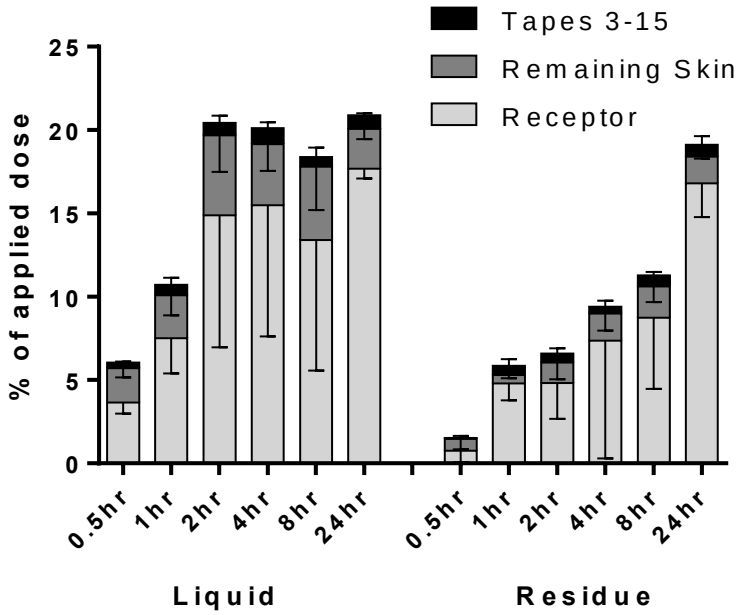

Figure 20 - Stacked bar chart showing skin uptake and absorption of TXP after 24 hours when the skin surface had been decontaminated after exposure periods of 0.5, 1, 2 ,4, 8 and 24 hours (mean ± SD; n = 3-7)

A 2-way analysis of variance (ANOVA) of these results shows that the time of decontamination had a significant impact on the % of the applied amount of TXP absorbed ($p < 0.001$), as did the application type (liquid versus residue) ($p < 0.0001$). The interaction between the two variables, however, was not significant ($p = 0.3$).

The total uptake and absorption of TXP from the spray dilution was significantly reduced when the skin surface was cleaned within 1 hour of exposure. However, if decontamination was delayed to 2 hours post-exposure or longer, then the % of the applied 'dose' taken up and/or permeated was unchanged (at about 20%). This finding is consistent with the results in Figure 19(a) which shows that the majority of TXP permeation across the skin had occurred within 4 hours. In contrast, the uptake and absorption of TXP permeation from the residue increased progressively with the exposure period; in fact, when the exposure

period was 24 hours, the uptake and absorption of TXP from the residue was not significantly different to that from the spray dilution.

Taken together, the results from this research permit three broad conclusions to be drawn. First, with the optimised method employed, residue transfer to the skin can be achieved reliably and reproducibly, with good efficiency, so that valid comparisons are possible between AIs of different physicochemical properties delivered from the same vehicle. Second, it is evident that the uptake and absorption of pesticide from a dried residue is generally less than when the same chemical is presented to the skin as a spray dilution; this general behaviour, which had been reported previously for one compound only[129], seems to hold for pesticides differing quite widely in their physicochemical properties. Despite a large range in the predicted maximum fluxes of the four chemicals across the skin (Table 4), the difference in the absolute quantities taken up and penetrated between the most and the least absorbed only ranged from 1.8 to 3.1-fold between spray dilution and residue applications. Third, the decontamination experiments with TXP reveal that, following exposure to the spray dilution, it is important to clean the skin within 1 hour to significantly reduce potential systemic exposure; indeed, removing material from the surface at 30 minutes post-exposure can reduce dermal uptake by 4-fold. With respect to exposure to a dried residue, the data indicate that the sooner decontamination is performed, the greater the reduction in exposure is achieved. For example, cleaning the skin after 30 minutes of contact with the residue reduces potential systemic exposure by a factor of 12 to that resulting from continual contact for 24 hours. Even if the residue-contaminated skin is only washed at the end of an 8-hour working day, the potential systemic exposure (relative to that at 24 hours) is halved. As this study was performed *in vitro* using pig skin, conclusions about the *in vivo* human scenario should be drawn with caution, however, this work provides further validation of the methodology for residue application and some insight in to how these residues may be absorbed in a re-entry scenario. Further work should focus

on validating the conclusions drawn *in vitro* using human skin from several donors and *in vivo* in humans using a model compounds such as ibuprofen.

4.5.4 Further Discussion

The results from this study found that AI had a significant effect on total absorption, from both liquid and residue application. This is not a new observation of course, however, the differences observed between the compounds were not as large as perhaps may have been expected, taking in to account their varying physicochemical properties.

The predicted maximum flux values of the four compounds were more than 30-fold different from the highest, 1.02 μg/cm^2/h for PPZ, to the lowest, 0.032 μg/cm^2/h for CLF. However, the difference in observed total absorption between the highest and lowest was only around 2.5-fold when applied as a spray dilution and 3.3-fold as a residue. These values did however predict the correct rank order of absorption efficiency for these compounds.

Potential reasons for this difference may be that these predicted values are based on absorption from an infinite dose of a saturated aqueous donor, which was not the case for the present study. By considering the specific examples of TXP and CLF flux, it is possible to follow how these factors may affect total absorption.

Firstly, the concentration of AI used was 1 mg/ml; for TXP this value is 10-fold lower than its maximum aqueous solubility, whereas for CLF this value is far higher than its maximum aqueous solubility (0.004 mg/ml). It is not known to what extent the presence of the solubility enhancing excipients would increase the solubility of either AI in the spray dilution, but it is clear that TXP was present at a concentration significantly below saturation in the vehicle, which would negatively impact its partitioning in to the SC. Conversely, CLF was present at a concentration significantly above its maximum aqueous solubility.

Secondly, only 30 μL of this solution was applied to the skin, representing a finite dose scenario i.e., dose depletion could become a factor. This would likely have a more significant impact on TXP, as it is absorbed faster than CLF.

Finally, from such a small volume, as time progresses, volatile components of the vehicle would evaporate away or pass in to the skin, changing its thermodynamic activity. This could result in a temporary increase in flux due to concentration of the AI in the vehicle, however, as seen in Figure 27 in Chapter 6, most of the water has evaporated away just 1 hour post-application. This would then leave a deposition on the skin surface that is not dissimilar to the dried residue being investigated. Consequently, dissolution from this 'residue' is now necessary before the AI can be absorbed further. The more lipophilic compound, CLF, at this point would likely partition in to the SC more effectively than TXP which is more hydrophilic.

Thus, overall, it is not unexpected that observed fluxes were significantly lower than the predicted values. The examples above also show how the physicochemical properties of the compounds can modulate these effects, explaining why only a 2.5-fold, rather than 30-fold, difference was observed between the compounds.

Further to the results shown in Paper 2, Figure 21 (a to f) shows cumulative TXP in the receptor for each exposure time investigated. The largest difference between TXP absorption from liquid and residue was seen when decontamination occurred at 0.5 hours post-exposure (Figure 21 (a)). In this case, TXP was absorbed 4-fold more when applied as a spray dilution compared to when applied as a residue. The longer the exposure time, in general, absorption from the two application types tended towards each other, up to 24-hour exposure where there was no significant difference between the two (Figure 21 (f)).

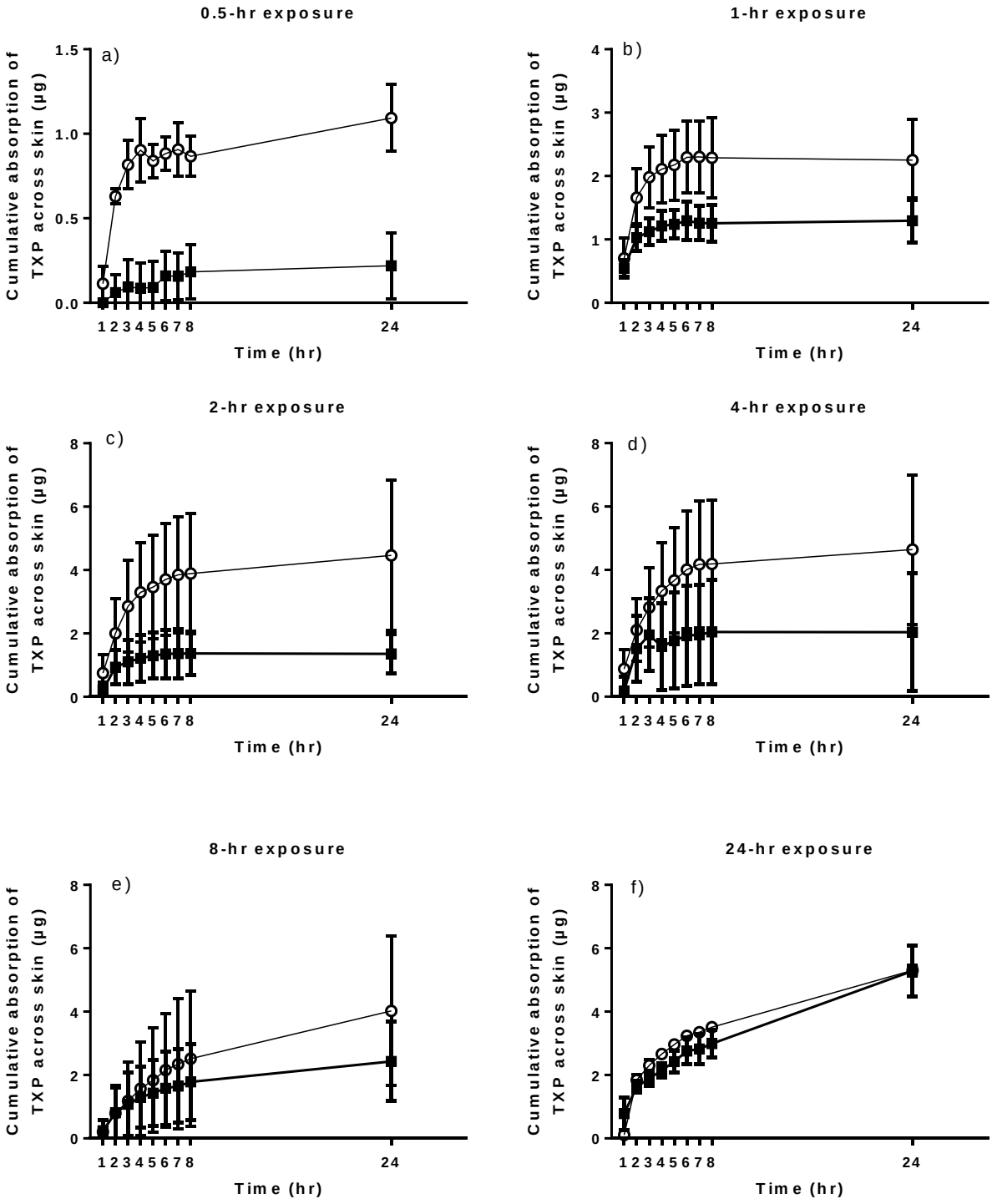

Figure 21 – Cumulative amount of TXP in Receptor (µg) from exposure times of (a) 0.5-hr (b) 1-hr (c) 2-hr (d) 4-hr (e) 8-hr (f) 24-hr. Open = Liquid application Solid = Residue

If it is assumed that these results are representative of the *in vivo* human scenario (discussed further in Chapter 6), these graphs highlight how decontamination of the skin as early as possible is important if exposed to TXP in any form. If exposed

to a spray dilution of TXP it would be crucial to decontaminate the area within the first hour to reduce systemic exposure. A similar observation can be made for exposure to TXP residue, washing early can reduce exposure by an order of magnitude. Additionally, these results show that even if a worker is not able to wash until after the working day has finished, 8 hours after first contact with TXP residue, this decontamination can still significantly reduce systemic exposure.

This observation could also influence risk assessment. The current approach for re-entry workers assumes a fixed exposure time of 8 hours for the entire daily potential dermal exposure calculated. However, in reality, the worker would not be exposed to all of the daily dose at once, but to small amounts throughout the day. It may therefore be possible to take this exposure pattern into account to procure more realistic systemic exposure estimates.

The results from paper 2 have further validated the methodology for application of pesticide residues *in vitro*. Residue transfer was consistent throughout the study. Although the transfer procedure had been changed slightly, transfer of TXP was similar to that described in Paper 1 at 22.5 ± 4.3μg. PPZ, CLF and DFZ transfers were 24.4 ± 1.3 μg, 30.0 ± 2.6 μg and 29.0 ± 3.4 μg, respectively. The fact that all four compounds had similar transfer efficiencies despite varying physicochemical properties suggests that the formulation may play a more important role in transfer than the AI. So far, all experiments have been with compounds in the same emulsifiable concentrate formulation. Pesticide products come in many different formulations and in order to fully validate the methodology, it should be tested on more than one formulation type. Different formulations are likely to have a significant effect on the nature of the dried residue even when the same AI is used. Additionally, all experiments to date have been performed at one dose level, 30 μg (15 μg/cm^2), in order to validate the methodology and investigate how different 'doses' could affect the absorption of dried residues, absorption should be measured from different loading doses.

5 PAPER 3 - EFFECTS OF FORMULATION AND LOADING ON DERMAL ABSORPTION OF PESTICIDE RESIDUES

The experiments carried out in Paper 2 have served to further validate the methodology by demonstrating its reproducibility using various active ingredients. However, all experiments thus far have been from a single EC formulation and at a single dose level. The research presented in this section aimed to investigate two compounds, CLF and TXP, from three commercial formulations and various loading doses, with a view to further validate the methodology and probe how these varied experimental conditions may affect absorption.

Aims:

- Investigate the effect of formulation on dermal absorption of TXP and CLF from both spray dilution and residue applications.
- Investigate dermal absorption of TXP and CLF from various loading doses, and determine if absorption from spray dilution and residue applications is affected.

5.1 Contributions

This manuscript was submitted to 'Chemical Research in Toxicology' in September 2017, all practical work described was carried out by the first author. The manuscript was written by the first author, and edited and approved by all authors.

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5.2 Abstract

Dermal risk assessment for re-entry workers, who enter a field after pesticide application to perform a task such as crop inspection or harvest involves exposure to a dried residue of the spray dilution. In this research, a robust and reproducible *in vitro* methodology, designed by this group, is used to measure absorption of these residues *in vitro* and compared against the conventional approach whereby dermal uptake and absorption is determined by testing liquid forms of the pesticide. Experiments have been conducted on liquid spray dilutions and their dried residues of two physicochemically distinct pesticides, trinexapac-ethyl (TXP) and clodinafop propargyl (CLF), formulated in two emulsifiable concentrate formulations and one wettable powder. As a control, deposition from a simple solvent was also examined. Furthermore, from one of the formulations, the effect of 'loading' (dose per unit area) on skin uptake and absorption of the two pesticides was quantified for both the liquid spray dilution and the dried residue. Overall, the results presented indicate that dermal exposure of re-entry workers to dried pesticide residues is determined by the chemical's properties, the formulation used, and the amount contacting the skin. It appears that dermal uptake and penetration of a pesticide from dried residues is typically less than that for liquid spray dilutions, and that penetration of the pesticide across the skin can be subtly influenced by excipients in the formulation.

5.3 Introduction

The amount of pesticides used globally each year is measured in millions of tonnes[131]. These chemicals are usually designed to target specific organisms but negative effects can also be elicited in humans when inadvertent exposure occurs. Consequently, before being licensed for use, each pesticide product must undergo a risk assessment involving different, potential routes of exposure, such as oral, inhalation and dermal[7]. Dermal exposure can occur via accidental spillage of a product on the skin during, for example, mixing and loading procedures[8]. Skin contact with contaminated surfaces is another common exposure scenario, particularly for re-entry workers, to whom pesticide transfer from treated plants and fruits is a potential hazard during the course of their daily work.

The European Food Safety Authority (EFSA) exposure assessment[7,8] for re-entry workers estimates the Potential Dermal Exposure (PDE), in μg of active substance per day, using the following relationship:

PDE = DFR x TC x T

DFR is the Dislodgeable Foliar Residue (in μg of active substance per cm^2 of leaf surface) and corresponds to the amount of pesticide that can potentially be dislodged and transferred to the skin. The DFR depends on several factors including application rate and crop type. In an initial risk assessment, the DFR is typically unknown, and a conservative, default value of 3 $\mu g/cm^2$ per kg AI applied per hectare is used in the EU.

TC is the Transfer Coefficient (with units of cm^2/h), which measures the extent of contact with the foliage and is specific to the crop type and task being performed. There are a number of sources of TC values, such as the EUROPOEM II database[16]. Generally, risk assessments assume that the arms, body and legs of a field worker are covered while the hands are not[7]. EU TC values for harvesting vary from a relatively low 2,500 cm^2/h for vegetables up to as high as 10,100 cm^2/h for grapes.

T is the duration of exposure (in hours per day); for instance, 2 h/day is considered typical for crop inspection, 8h/day for harvest. Examples of exposures calculated using this equation are given below, one representing a worst-case high exposure, the other corresponding to a relatively low exposure.

$PDE_{grape\ harvest}$ = 3 µg/cm² x 10,100 cm²/h x 8 h/day = 242 mg/day (assuming 1 kg AI/ha)

$PDE_{vegetable\ harvest}$ = 0.03 µg/cm² x 2,500 cm²/h x 8 h/day = 0.6 mg/day (assuming 0.01 kg AI/ha)

Dividing the PDEs by 820 cm², the EFSA default value for the surface area of the hands, yields estimated doses of 296 and 0.7 µg/cm²/day, respectively. It should be noted that the above calculations represent a very simplistic model, but they serve to give a general estimate of exposure levels.

Once the PDE has been calculated, systemic exposure is then estimated using a percentage absorption factor, which is currently determined experimentally by measuring the skin penetration of the pesticide from the concentrate product and spray dilutions thereof, typically *in vitro*. These different formulations are tested because it has been found that dermal loading can affect the percentage absorption (and, generally as dermal loading increases, the fraction absorbed decreases)[65-67]. However, with respect to skin contact with a contaminated plant surface, exposure occurs to a so-called 'dried residue' of the pesticide spray dilution; 'dried', in this case, implies only that the volatile components of the formulation (in particular, water) have evaporated away. Still, no matter the final composition of the 'dried' residue, the uptake of a pesticide from this film may well be different to that from a liquid spray dilution.

In terms of assessing systemic exposure to a pesticide from a dried residue, it is important that the residue is formed from an appropriate formulation, the uptake from which may differ to that from a simple aqueous solution[108, 109]. This may be due to the presence of non-volatile solvents, for example, which can influence the partitioning and/or diffusion of pesticide in the stratum corneum (SC), the

outermost, and principal barrier layer of the skin[107, 112, 113]. Surfactants are also common ingredients in pesticide formulations, and these chemicals may also comprise part of a 'dried' residue. Consequently, they may facilitate the dissolution of a sparingly soluble AI and enable its solubilisation in the skin as well (and even, perhaps, facilitate penetration across the SC[114-116]). EFSA has recognised the importance of the potential effect of a formulation on dermal uptake[8] and requires a new risk assessment to be carried out when a single component of a product is changed by more than 25% w/v.

An improved experimental methodology has been reported for the measurement of pesticide dermal absorption from a dried residue *in vitro*[129]. It was shown that the skin uptake and absorption of trinexapac-ethyl from the spray dilution of a naphtha-based emulsifiable concentrate (EC) formulation was significantly higher than that from its dried residue. This initial proof-of-concept, however, involved one pesticide, at a single dermal loading, and applied from one formulation. The research described in the present study aims at a broader scope so that firmer conclusions may be drawn about the effects of formulation and dermal loading on the skin penetration of pesticides from dried residues.

5.4 Materials & Methods

Two pesticides were considered: (a) clodinafop-propargyl (CLF), a herbicide with a log(octanol-water partition coefficient)(log P) of 3.9, molecular weight = 350 Daltons, and aqueous solubility of 4 μg/mL[132]; and (b) trinexapac-ethyl (TXP), a plant growth regulator having log P = -0.29, molecular weight = 252 Daltons, and water solubility = 10.2 mg/mL[132]. Spray dilutions of three commercially relevant formulations (see below) of the two pesticides were prepared. For TXP, a simple aqueous solution (1 mg/mL in distilled water) acted as a control. For CLF, which has a very low water solubility (0.004 mg/mL), an aqueous suspension was flocculent and, to create a control residue of this chemical, a 1mg/mL solution in acetone was used instead.

Static Franz diffusion cells, having a receptor volume of 7.4 mL and area of exposed skin of 2 cm^2, were used with porcine skin (dermatomed to a nominal thickness of 750 μm). The receptor solution was phosphate-buffered saline (pH 7.4) for TXP, and 0.5% w/v Volpo™ (Sigma-Aldrich Co., Gillingham, UK) in PBS for CLF. A positive displacement pipette, was used to apply 30μl of each pesticide spray dilution to the skin. Samples of the receptor solution were taken hourly from 2 to 8hrs and at 24 hours for TXP; at 8 and 24 hours only for the more poorly penetrating CLF. For both chemicals, the skin was 'decontaminated' at 8 hours: 100μl of a 0.1% w/v soap solution was applied to the skin surface followed by immediate swabbing with two cotton buds.

Application of pesticide residues to the skin followed a previously described and validated methodology:[129] 40μl of the spray dilution was applied to a steel disc (SPM specimen discs, TAAB Laboratories Equipment Ltd., Aldermaston, U.K.) and allowed to dry for 24 hours to form a residue, which was then transferred to the skin with a standardised procedure[129]. Subsequently, the amount of pesticide remaining on the disc was quantified to determine precisely the 'dose' applied for each replicate.

The pesticide formulations examined were: [1] An emulsifiable concentrate formulation (EC-A) (Syngenta, Jealott's Hill, UK) that contained naptha as the primary solvent and castor oil, calcium dodecylbenzene sulphonate and tristyrylphenol ethoxylated as emulsifiers. The pesticide concentration in EC-A was 100mg/mL. [2] A second emulsifiable concentrate formulation (EC-B) (also supplied by Syngenta at a pesticide concentration of 100 mg/mL) with 1-phenylethan-1-one as the primary solvent, the same emulsifiers as EC-A, and the surfactant, oleic acid methyl ester. Both EC formulations were diluted 100-fold in water to provide nominal spray dilution concentrations of the active ingredient of 1 mg/ml. [3] A wettable powder (WP) formulation based on the filler, kaolin, and also containing a dispersant, lignin sodium sulphate, and a wetting agent, butylnaphthalenesulphonic acid salt was also tested. The pesticide concentration in the WP was 15% w/w. To prepare a spray dilution at a concentration of 1 mg/mL, 667 mg were made into a paste, passed through a sieve (Endecotts, London, UK) of aperture 125 μm, and then made up to 100 mL in water. As the resulting formulation was a suspension, thorough mixing by vortex was performed before each use.

In addition, both TXP and CLF were investigated as various 'dose' levels. 30, 70 and 120 μL of TXP EC-A spray dilution were applied. The corresponding residue discs were loaded with 40, 100 and 180 μL of the same spray dilution to match the dose levels. 30, 70, 120 and 160 μL of CLF EC-A spray dilution were applied and residue discs were loaded with 40, 100, 180 and 250 μL.

5.5 Results

CLF and TXP were applied to the skin from three commercial formulations, as a liquid spray dilution and as a residue. TXP was also applied from an aqueous solution and from its relative dried residue; CLF was additionally applied as a residue after evaporation of an acetone solution of the compound.

The amount of CLF residue transferred to the skin surface depended on the formulation used (one-way analysis of variance, $p < 0.01$). The average amounts applied were: 22.6 (± 1.8) μg for EC-A, 26.2 (± 1.3) μg for EC-B, 35.2 (± 1.96) μg for WP, and 17.9 (± 3.3) μg for the AI deposited from acetone. These quantities were used when calculating percentages of the applied dose absorbed. No CLF was detected in the receptor solution from any application. The total absorption of this pesticide was therefore considered as the sum of the amounts of CLF in stratum corneum tape-strips 3-15 (assuming CLF in tapes 1 and 2 to be lost *via* desquamation) and in the remaining skin tissue. The results are shown in Figure 22.

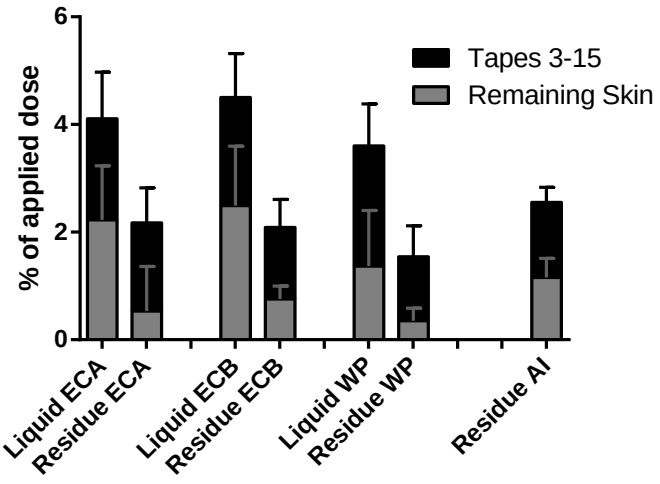

Figure 22 - Stacked bar chart showing the skin uptake of CLF at 24 hours after liquid and residue applications from different formulations (mean ± SD; see Tables 9 and 10 for number of replicates).

Formulation type had no significant impact on CLF uptake and absorption for the liquid application (Table 9). There was also no difference observed in total CLF uptake and absorption from dried residues (Table 10). However, when the estimated amount applied for each formulation was taken in to account, % uptake/absorption was significantly different between formulations (1-way ANOVA, $p < 0.01$). Formulation also influenced the amount of CLF recovered from the stratum corneum on tapes 1 and 2; notably, if these quantities were included in the % uptake/absorption, no significant difference was observed

between formulations. This information is potentially important because the amount of pesticide recovered from the first two stratum tape-strips was greater than that recovered from the remaining tapes and skin combined. Hence, when one discards this mass from the % amount taken up and absorbed, the effect is substantial and is quite different than that found for TXP, which has been demonstrated to be absorbed across the skin much more quickly than CLF (see Table 11 and Table 12 below).

Table 9 - Skin uptake and absorption of CLF from liquid spray dilution application of three formulations (mean ± SD).

CLF formulation - liquid	**EC-A**	**EC-B**	**WP**
Number of replicates (n)	6	6	8
CLF applied (µg)	30	30	30
CLF recovered in swabs (µg)	21.70 ± 2.84	21.82 ± 1.89	24.67 ± 2.35
CLF disposition			
SC tape-strips 1-2 (µg)	1.25 ± 0.49	1.28 ± 0.40	1.78 ± 0.53
SC tape-strips 3-15 (µg)	0.54 ± 0.26	0.60 ± 0.24	0.67 ± 0.23
SC tape-strips 1-15 (µg)	1.80 ± 0.69	1.88 ± 0.46	2.45 ± 0.62
Remaining skin (µg)	0.67 ± 0.30	0.75 ± 0.33	0.41 ± 0.31
Receptor phase at 24 hr (µg)	< LOQ	< LOQ	< LOQ
Total CLF uptake/absorption (µg)[#]	1.21 ± 0.43	1.35 ± 0.30	1.08±0.34
% uptake/absorption of CLF applied	4.04 ± 1.42	4.50 ± 1.01	3.60 ± 1.13

< LOQ = below the limit of quantitation of CLF.

[#]Sum of (SC tape-strips 3-15) + (Remaining skin)

Table 10 -Skin uptake and absorption of CLF from residue application of three formulations and acetone vehicle. (mean ± SD).

CLF formulation - residue	**EC-A**	**EC-B**	**WP**	**Acetone**
Number of replicates (n)	6	4	8	5
CLF applied (µg) *	22.58 ± 1.81	26.16 ± 1.33	35.23 ± 1.96	17.87 ± 3.26
CLF recovered in swabs (µg) *	18.16 ± 2.46	19.68 ± 1.41	32.00 ± 5.59	9.76 ± 2.66
CLF disposition				
SC tape-strips 1-2 (µg) †	0.48 ± 0.07	0.58 ± 0.17	1.08 ± 0.57	0.47 ± 0.13
SC tape-strips 3-15 (µg)	0.37 ± 0.14	0.35 ± 0.13	0.42 ± 0.20	0.24 ± 0.03
SC tape-strips 1-15 (µg) †	0.85 ± 0.17	0.93 ± 0.19	1.50 ± 0.72	0.71 ± 0.11
Remaining skin (µg)	0.13 ± 0.20	0.20 ± 0.07	0.12 ± 0.08	0.20 ± 0.05
Receptor phase at 24 hr (µg)	<LOQ	<LOQ	<LOQ	<LOQ
Total CLF uptake/absorption (µg)#	0.49 ± 0.09	0.55 ± 0.07	0.54 ± 0.15	0.45 ± 0.07
% uptake/absorption of CLF applied *	2.17 ± 0.31	2.09 ± 0.29	1.54 ± 0.44	2.55 ± 0.54

*Significantly different ($p < 0.01$) between formulation type (1-way ANOVA).

†Significantly different ($p < 0.05$) between formulation type (1-way ANOVA).

< LOQ = below the limit of quantitation of CLF.

#Sum of (SC tape-strips 3-15) + (Remaining skin)

Transfer of TXP residue was not significantly different between the three commercial formulations: 27.1 (± 1.5) µg for EC-A, 29.1 (± 3.1) µg for EC-B, 27.3 (± 5.7) µg for WP. However, the AI transferred from residue formed from an aqueous solution was considerably less (11.34 (± 0.96) µg). A 2-way ANOVA revealed that skin uptake and absorption of TXP was significantly different between formulations and application type ($p < 0.001$). There was also an

interaction between these two variables ($p < 0.01$). TXP was absorbed significantly less (relative to the liquid spray dilutions) when applied as a residue from EC-A and EC-B, but not from WP (Figure 23, Table 11 and Table 12). Permeation of TXP from the spray dilutions was significantly different between formulations. On the other hand, the amounts of TXP taken up in the SC and the remaining skin tissue were similar between the formulations.

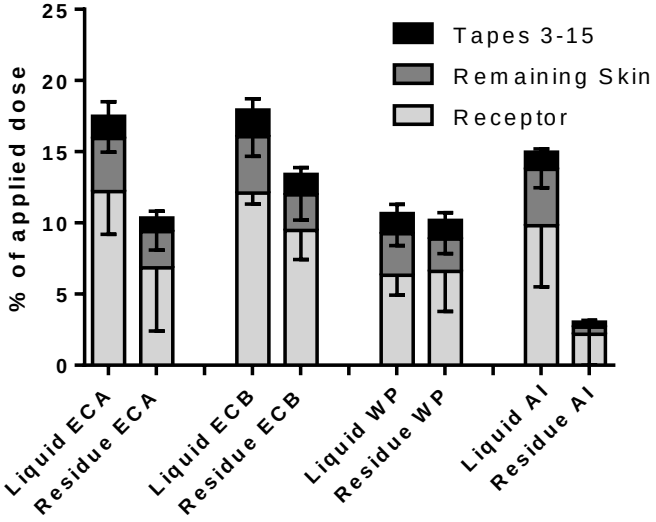

Figure 23 -Stacked bar chart showing the skin uptake and absorption of TXP at 24 hours after liquid and residue applications from different formulations (mean ± SD; see Tables 11 and 12 for number of replicates).

In Table 12, the statistical analysis presented was performed on the results from the three commercial formulations. The data of TXP absorption from the aqueous solution residue was not included because of the much smaller quantity that was able to be transferred; clearly, the amounts of pesticide found to have been taken up by, or absorbed through, all the skin 'compartments' were considerably smaller than from the commercial formulations. The total % 'dose' exposure was also significantly less. With respect to the three commercial formulation residues, there was notably no significant difference between them for any of the measured quantities.

Table 11 - Skin uptake and absorption of TXP from liquid spray dilution application of three formulations and from an aqueous solution (mean ± SD).

TXP formulation - liquid		**EC-A**	**EC-B**	**WP**	**Aq. solution**
Number of replicates		5	4	5	4
TXP applied (µg)		30	30	30	30
TXP recovered in swabs (µg) *		18.84 ± 1.77	17.07 ± 1.95	20.27 ± 0.80	13.13 ± 0.76
TXP disposition					
SC tape-strips 1-2 (µg)		1.10 ± 0.33	0.82 ± 0.12	0.97 ± 0.13	1.12 ± 0.47
SC tape-strips 3-15 (µg)		0.55 ± 0.38	0.56 ± 0.24	0.43 ± 0.20	0.36 ± 0.07
SC tape-strips 1-15 (µg)		1.65 ± 0.70	1.39 ± 0.28	1.40 ± 0.29	1.48 ± 0.53
Remaining skin (µg)		1.09 ± 0.29	1.18 ± 0.41	0.87 ± 0.25	1.18 ± 0.38
Receptor phase (µg)	2 hr†	1.07 ± 0.35	0.66 ± 0.46	0.50 ± 0.13	0.80 ± 0.39
	3 hr*	1.72 ± 0.52	1.38 ± 0.09	0.76 ± 0.15	1.25 ± 0.64
	4 hr*	2.14 ± 0.68	1.73 ± 0.18	0.98 ± 0.20	1.57 ± 0.81
	5 hr†	2.52 ± 0.88	1.91 ± 0.21	1.16 ± 0.25	1.75 ± 0.93
	6 hr†	2.78 ± 0.90	2.20 ± 0.23	1.27 ± 0.25	2.00 ± 1.04
	7 hr†	2.93 ± 0.92	2.24 ± 0.25	1.27 ± 0.20	2.14 ± 1.07
	8 hr†	3.09 ± 1.02	2.51 ± 0.26	1.35 ± 0.25	2.26 ± 1.12
	24 hr*	4.04 ± 1.09	3.63 ± 0.23	1.90 ± 0.42	2.94 ± 1.29
Total TXP uptake/absorption # (µg) *		5.69 ± 0.90	5.38 ± 0.79	3.20 ± 0.78	4.49 ± 1.16
% uptake/absorption of TXP applied *		18.95 ± 2.99	17.92 ± 2.63	10.65 ± 2.59	14.96 ± 3.85

*Significantly different ($p < 0.01$) between formulation type (1-way ANOVA).

†Significantly different ($p < 0.05$) between formulation type (1-way ANOVA).

#Sum of (SC tape-strips 3-15) + (Remaining skin) + (Receptor phase at 24 hr).

Table 12 - Skin uptake and absorption of TXP from residue application of three formulations and from aqueous solution (mean ± SD).

TXP formulation - residue		**EC-A**	**EC-B**	**WP**	**AI**
Number of replicates		5	7	9	3
TXP applied (µg)		27.07 ± 1.46	29.12 ± 3.09	27.28 ± 5.72	11.34 ± 0.96
TXP recovered in swabs (µg)		18.87 ± 3.54	14.28 ± 2.11	15.74 ± 3.41	3.34 ± 0.95
TXP disposition					
SC tape-strips 1-2 (µg)		0.66 ± 0.24	0.85 ± 0.32	1.16 ± 0.84	0.19 ± 0.10
SC tape-strips 3-15 (µg)		0.29 ± 0.14	0.41 ± 0.14	0.37 ± 0.20	0.04 ± 0.01
SC tape-strips 1-15 (µg)		0.95 ± 0.38	1.26 ± 0.42	1.54 ± 0.93	0.23 ± 0.09
Remaining skin (µg)		0.76 ± 0.40	0.75 ± 0.60	0.61 ± 0.29	0.06 ± 0.11
Receptor Phase (µg)	2 hr	0.72 ± 0.89	0.69 ± 0.40	0.71 ± 0.46	< LOQ
	3 hr	0.99 ± 1.06	1.06 ± 0.39	0.95 ± 0.55	< LOQ
	4 hr	1.23 ± 1.02	1.26 ± 0.43	1.07 ± 0.61	< LOQ
	5 hr	1.35 ± 1.09	1.46 ± 0.46	1.20 ± 0.62	< LOQ
	6 hr	1.49 ± 1.26	1.68 ± 0.56	1.31 ± 0.68	< LOQ
	7 hr	1.56 ± 1.24	1.83 ± 0.55	1.36 ± 0.68	0.06 ± 0.11
	8 hr	1.64 ± 1.26	2.00 ± 0.58	1.42 ± 0.72	0.09 ± 0.15
	24 hr	2.27 ± 1.34	2.76 ± 0.66	1.86 ± 0.88	0.25 ± 0.25
Total TXP uptake/absorption (µg)[#]		3.32 ± 1.33	3.92 ± 0.99	2.84 ± 1.00	0.35 ± 0.26
% uptake/absorption of TXP applied		12.18 ± 4.53	13.40 ± 2.79	10.30 ± 2.70	3.04 ± 2.26

'AI' was not included in statistical analysis

[†]Significantly different ($p < 0.05$) between formulation type (1-way ANOVA).

[#]Sum of (SC tape-strips 3-15) + (Remaining skin) + (Receptor phase at 24 hr).

In a further set of experiments, the skin uptake and penetration of CLF and TXP in EC-A were determined as a function of the applied quantities of the pesticide following both liquid spray dilution and residue applications. From the former, for CLF, 30, 70, 120 and 160 µg were deposited on the skin (2 cm^2) while the corresponding exposures from the residues were 23.9 (± 2.6), 67.2 (± 2.5), 130.5 (± 7.9) and 169.4 (± 3.0) µg, respectively. For TXP, three liquid and residue exposures were considered, with 30, 70 and 120 µg being applied from the former, and 25.8 (± 1.5), 66.9 (± 3.0) and 119.9 (± 7.9) µg from the latter.

The total skin uptake and absorption of CLF increased linearly with increasing quantity of the chemical applied for both the liquid and residue applications (Figure 24 (a); r2 = 0.97 and 0.94, respectively). In terms of the % 'dose' absorbed, a 2-way ANOVA revealed that, while more CLF was absorbed from the liquid, there was no significant effect of dose loading on either method of application (Figure 24 (b)). There was, however, a significant interaction between the two variables (i.e., the nature of the application and the skin 'loading'). Interestingly, when the quantities of CLF recovered in the first two stratum corneum tape-strips were included in the total skin uptake and absorption calculation, the 2-way ANOVA found that the chemical loading did have a significant effect of the % 'dose' absorbed, especially for the liquid application (Figure 24 (c)). The complete dataset is provided in the attached appendix.

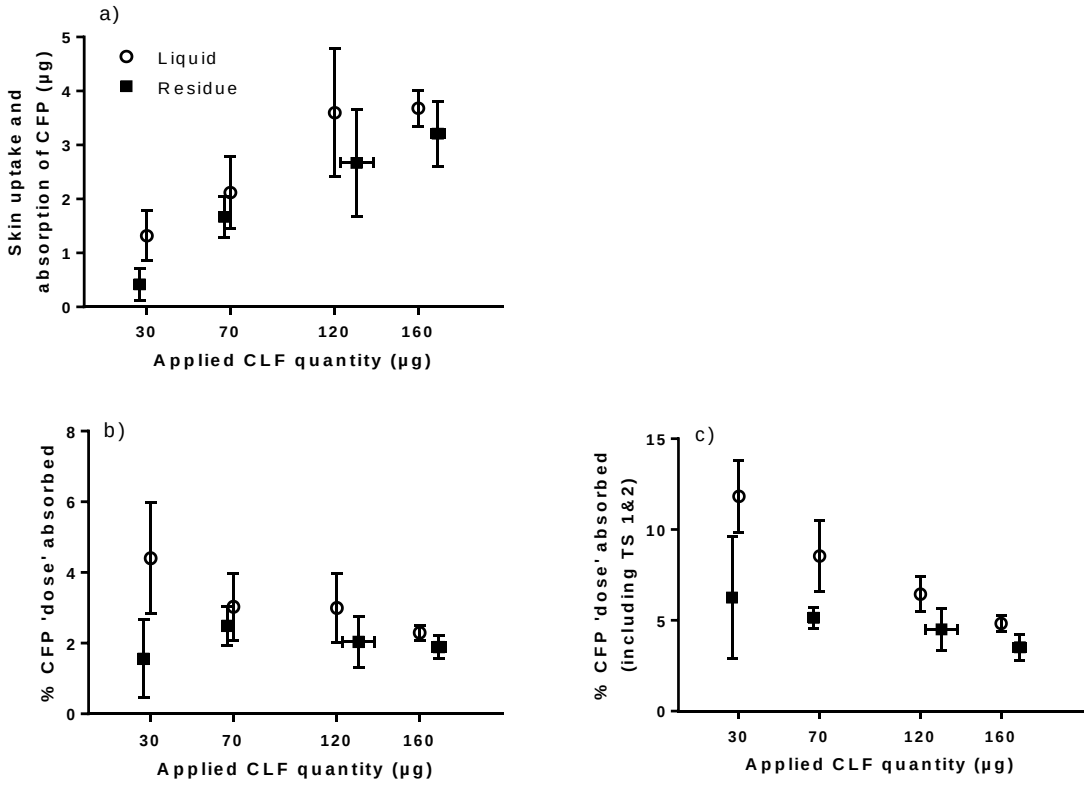

Figure 24 - CLF uptake and absorption into the skin (mean ± SD; n = 6 - 13) at 24 hours following 8-hour liquid and residue applications as a function of chemical loading ('applied dose') expressed (a) as the absolute quantity (mass), (b) as the % of the applied 'dose', and (c) as in (b) but including the pesticide recovered in the first two stratum corneum tape-strips (TS 1&2).

The total skin uptake and absorption of TXP also increased linearly with increasing quantity of the chemical applied for both the liquid and residue and applications (Figure 25 (a); r^2 = 0.94 and 0.82, respectively). In terms of the % 'dose' absorbed, there was no significant effect of dose loading for either method of application (Figure 25 (b)).

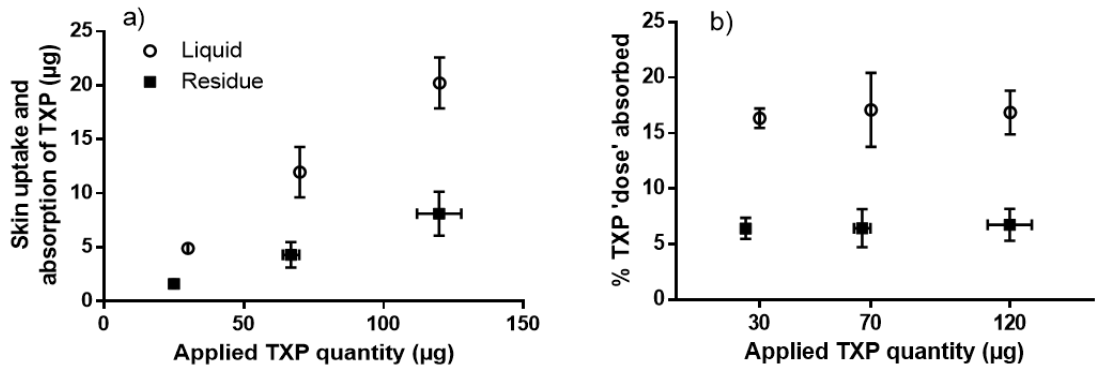

Figure 25- TXP uptake and absorption into the skin (mean ± SD; n = 4) at 24 hours following 8-hour liquid and residue applications as a function of chemical loading ('applied dose') expressed (a) as the absolute quantity (mass), and (b) as the % of the applied 'dose'.

The cumulative permeation of TXP into the receptor phase over time is shown in Figure 26 as a function of applied formulation and 'dose'. There was significantly less permeation from the residue at all loadings. Notably, TXP penetration from the highest residue loading only matched that from the lowest liquid 'dose', despite a nearly 4-fold difference in the quantities applied. The rates of TXP absorption can be inferred from the temporal change in the slopes of the lines in Figure 26. From the spray dilutions, the rates were clearly higher at the earlier times (especially up to 4 hours), before slowing to an approximately constant rate thereafter. In contrast, the rate of pesticide penetration from the residues was much steadier over the entire exposure period, even after 'decontamination' of the skin at 8 hours. The rates of TXP transfer across the skin between 8 and 24 hours were 'dose' dependent for both liquid and residue applications but, at each

loading, there was no significant difference between the rates for the two different applications (see Table 13).

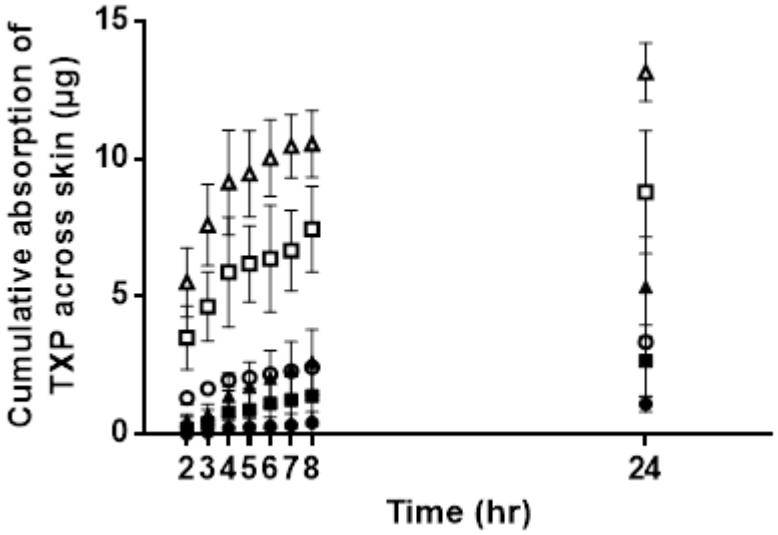

Figure 26 - Cumulative permeation of TXP (mean ± SD; n = 3 - 9) into the receptor phase over time as a function of applied formulation (open symbols = liquid; closed symbols = residue) and 'dose' (high = triangles; intermediate = squares; low = circles).

Table 13- Rates of TXP (mean ± SD n = 3 - 9) permeation across the skin as a function of 'dose'.

TXP application and loading[a]	**Low**		**Intermediate**		**High**	
	Liquid	Residue	Liquid	Residue	Liquid	Residue
0 – 4 hr (µg/hr)	0.50 ± 0.06	0.06 ± 0.07	1.48 ± 0.50	0.20 ± 0.20	2.28 ± 0.48	0.34 ± 0.18
4 – 8 hr (µg/hr)	0.12 ± 0.07	0.05 ± 0.04	0.39 ± 0.13	0.15 ± 0.07	0.35 ± 0.27	0.31 ± 0.13
8 - 24 hr (µg/hr)	0.06 ± 0.01	0.04 ± 0.01	0.08 ± 0.06	0.08 ± 0.02	0.16 ± 0.02	0.17 ± 0.05

[a]The exact TXP loadings are given in the text

5.6 Discussion

This study was designed to shed further light on the impact of pesticide properties, formulation and skin loading on the potential systemic dose

following dermal exposure of re-entry workers to dried residues on crops, plants, fruit, etc. The investigation was designed to include parallel measurements using liquid spray dilutions containing the same pesticides, at similar loadings in the same formulations. This comparison is valuable as risk assessments for re-entry workers are typically based on this exposure scenario (i.e., to the liquid spray dilution) rather than on the more realistic contact with a dried residue.

Consistent with previous reports[129], for the two pesticides considered (CLF and TXP, which differed appreciably in their physicochemical properties), skin uptake and absorption was significantly lower from the dried residue for all formulations and loading doses studied. The only exceptions were for CLF when applied at high dermal loading and for TXP applied in a wettable powder (WP) formulation, where no difference between spray dilution and residue was observed. A key component of the WP used is kaolin powder, a material used in traditional medicine and in numerous skin-care products (e.g., face masks, cosmetics, and in skin barrier formulations). Here, it seems possible that adsorption of the pesticide to the surface of the kaolin particles may become the common, principal factor controlling 'release' of the chemical to the skin, regardless of its application as a spray dilution or as a dried residue.

Otherwise, the kinetics of TXP penetration across the skin reveal some insight into the physics associated with dermal exposure. From the liquid spray dilution, the initial rate of penetration (over, say, the first 4 hours) is more rapid, presumably reflecting a 'metamorphosis' of the formulation as the aqueous phase evaporates, driving the thermodynamic activity of the pesticide higher (and increasing flux). However, once the solvent has gone, TXP penetration slows down as the chemical would now have to undergo a dissolution step before it is able to commence diffusing across the skin. From the residue, on the other hand, TXP is being released under fairly constant conditions as the residue is already dry when the application begins. While there may be some outward movement of water from the receptor solution towards the skin surface (and this may help

with dissolving the pesticide), transepidermal water loss is probably insufficient to completely redissolve the TXP in the residue.

Support for this analysis of the physical events taking place is found in the fact that, over the 8-24 hour period of the diffusion cell experiment, the penetration rates of TXP from the liquid spray dilution and from the residue are essentially the same, suggesting their control by the same mechanism; a potential candidate may simply be the slow emptying of the pesticide from the stratum corneum into the receptor solution.

With respect to the specific impact of the formulations, it is first worth noting that the reproducibility of transfer of the residues was very good, no matter the vehicle or the loading used. The transfer of CLF was affected by the formulation type, whereas TXP was not, an unsurprising observation given the differences in the physical chemical properties of the two pesticides. Transfer of both pesticides was more efficient from the commercial formulations than from simple solutions of the chemical (water for TXP, acetone for CLF), reflecting, it may be surmised, the role of excipients (e.g., surfactants) in facilitating solubilisation of the active ingredients.

An additional, noteworthy formulation-related observation concerned the emulsifiable concentrates (EC). The components of these products differ by more than 25% w/v, meaning that EFSA guidance would not allow bridging between the dermal absorption values for these two formulations. However, the results obtained in this work reveal no differences in skin uptake and absorption from the two formulations for either of the two pesticides considered.

Finally, as far as the impact of pesticide loading is concerned, it is worth pointing out that the lowest liquid application of 30 μL (i.e., 15 μL/cm^2 given the area of skin used) is approximately the minimum volume needed to just cover the skin surface. Larger loadings, or volumes, do not therefore increase the area of contact of the formulation with the skin, but do provide a greater reservoir of the

penetrant. The increased loadings also deposit more of the solvent from the liquid spray dilutions and prolong, as a result (and, as was discussed before) the time before the 'metamorphosis' of the deposited material is complete. Again, as previously explained, this behaviour is much less, if at all, apparent with the residues for which solvent has evaporated. Regardless, no matter the loading, the skin uptake and absorption of pesticide over 24 hours never exceeded 30% of the applied 'dose'; that is, an insufficient depletion to account for the slowing permeation of the chemicals as the experiment proceeded.

In conclusion, the results of the research reported here, together with those from earlier work, reveal that the potential systemic dose after dermal exposure of re-entry workers to dried pesticide residues is affected by the nature of the active ingredient, the type of formulation from which it is applied, and its 'loading' on the skin. Overall, though, while the effects are generally subtle, it is clear that (a) skin uptake and penetration from dried residues is smaller than that for liquid spray dilutions, and (b) formulation components can impact upon the ultimate 'delivery' of the pesticide across the dermal barrier.

6 ADDITONAL RESULTS & DISCUSSION

6.1 Deposition of Nile Red on Skin from Liquid and Residue Applications.

The spray dilutions used within this thesis are 99% v/v water. This water acts to solubilise the AI and can play a crucial role in the absorption process. Therefore, when water has been allowed to evaporate away before application to the skin, as is the case for the residue, the ability of the compound to partition in to the SC can be affected. This section aims to look more closely at deposition from both spray dilutions and residues.

To visualise how an AI might be distributed on the skin surface, the flourescent dye Nile Red (NR) was used as a model compound. Nile Red's physicochemical properties (Log P, 3.4 and MW, 318) are comparable to that of CLF which has a Log P of 3.9 and MW of 350. NR has been used in skin permeation studies previously[133, 134].

10 mg of NR was dissolved in 100 μL of a blank EC-A formulation, and then made up to 10 mL in water to form a spray diltuon (1 mg/mL), matching the concentration of the pesticide solutions studied in previous chapters of this thesis.

In initial experiments, 30 μL of NR spray dilution was applied to pig skin mounted in a diffusion cell positioned beneath a camera (Veho Discovery VMS-004 Deluxe Microscope, Coventry, UK). Images were taken before application, immediately post-application, and then 20 and 60 minutes thereafter (Figure 27).

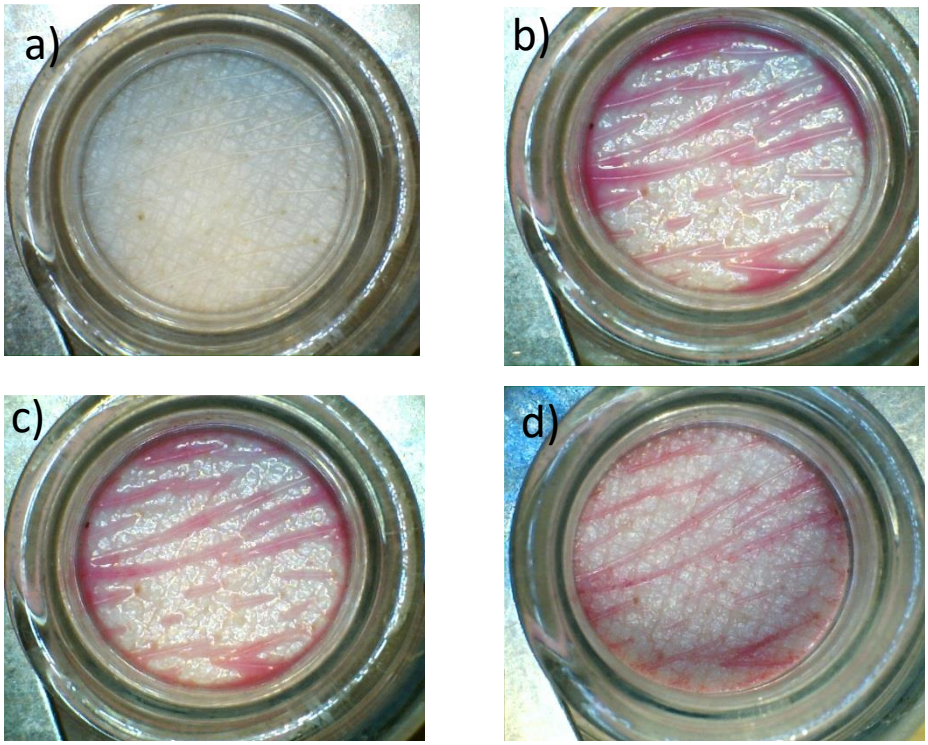

Figure 27 – Images taken (a) before application, (b) immediately post-application, and then (c)20 and (d) 60 minutes thereafter.

Although the solution was spread evenly across the surface of the skin, it is clear that NR is concentrated on the hairs and around the hair follicles (Figure 27 (b)).

There is a noticeably bright reflection of light from the skin surface immediately post-application of the spray dilution (Figure 27 (a)), that is still visible 20 minutes later (Figure 27 (b)); however, this has dissipated by 1 hour (Figure 27 (c)). Most likely, this is due to the initial presence of water on the skin surface that gradually evaporates and/or penetrates into the skin.

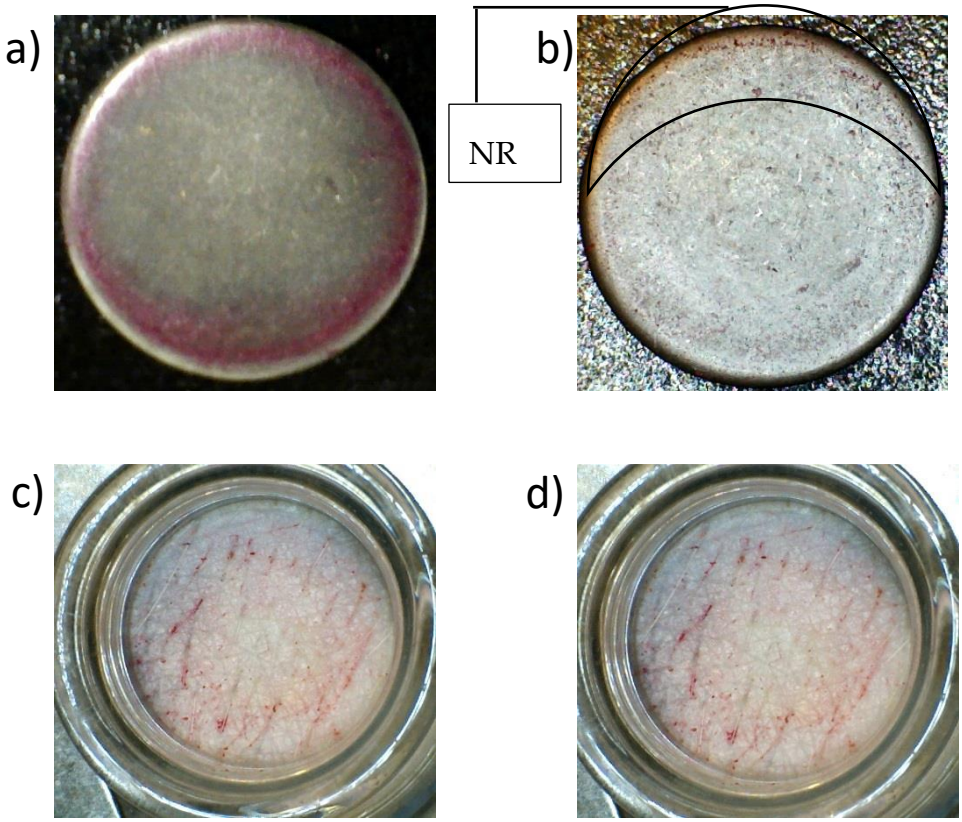

Figure 28 - Images of the AFM disc taken (a) before application to the skin and (b) immediately post-application. Images of the skin taken (c) immediately post-application and (d) 60 minutes thereafter.

40 μL of the 1 mg/mL NR dilution was then loaded onto an AFM disc and allowed to dry for 24 hours (Figure 28 (a)). As observed previously, NR was deposited around the edge of the disc in a 'coffee ring' formation. NR was then transferred to the skin using the protocol described in previous chapters of this thesis. Figure 28 (b) shows the disc post-application with some NR still remaining around the edges of the disc. Figure 28 (c) shows the skin surface immediately after application of the residue and, again, NR is predominantly localised around the hair follicles. After 60 minutes, no further change in NR disposition has occurred (Figure 28 (d)).

Subsequently, skin to which the NR spray dilution (Figure 29) and residue (Figure 30) had been applied was also examined under a light microscope (Motic BA210, Motic, Hong Kong) with a 5 Megapixel camera attachment (Moticam, Motic, Hong Kong).

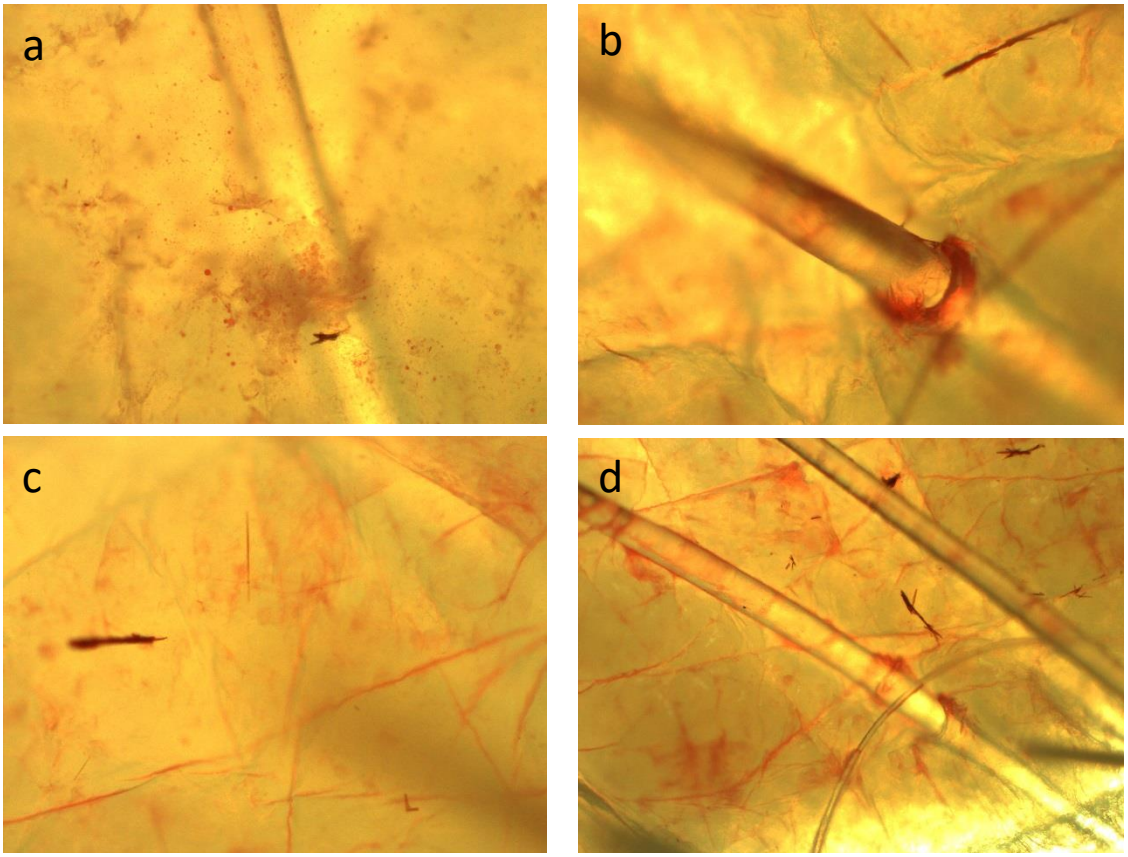

Figure 29 - Light microscope images of Nile Red Spray dilution on the surface of the skin taken (a) immediately post-application and (b, c & d) 24 hours post-application

Figure 29 (a) is an image of the skin surface immediately after application of 30 µL of the NR spray dilution solution. The dispersion of the oily NR in water is clearly localised around the hair follicle. Panels (b), (c) and (d) of Figure 29 were taken 24 hours later and show, respectively, accumulation of NR around the base of a hair follicle, the presence of NR 'trapped' in the crevasses of the skin surface, and the affinity of NR to the hair shaft.

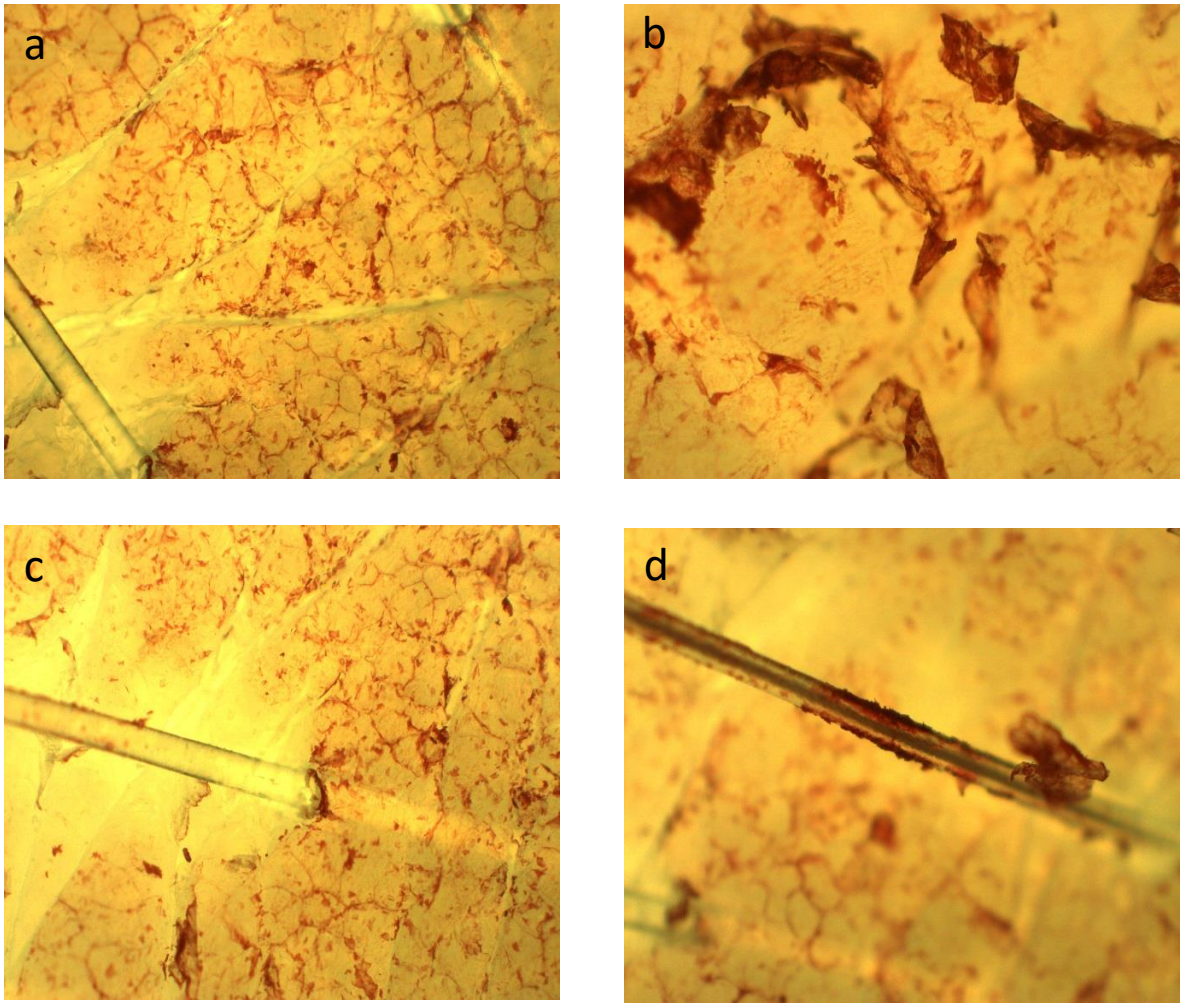

Figure 30 - Light microscope images of Nile Red residue on the surface of the skin taken immediately post-application

Figure 30 shows NR disposition on the skin immediately after application of a dried residue. A significant presence of NR in the crevasses of the skin is apparent, presumably because the rough topography of the surface dislodges the residue from the disc during the application process (panels (a) and (c)). NR seems to be distributed relatively evenly across the skin, except where a hair shaft appears to protect the skin below from exposure to the residue (see Figure 30 (c), for example). At higher magnification, (Figure 30 (b)), the presence of crystals of NR can sometimes be seen. As for deposition from the spray dilution, the affinity of NR to the hair shaft was also quite obvious when the compound was applied from a dried residue. (Figure 30 (d)).

Imaging the behaviour of NR when applied to the skin from liquid and residue highlighted some interesting differences between the two application types, it also demonstrated that hair follicles and fibres may be an important factor, although this effect could be specific to NR. It is probable however, that the effect of the hair fibre protecting the skin from transfer of residue would be present for other compounds and formulations. While the coverage of NR residue was generally quite good, these areas of no deposition could potentially contribute to diminished absorption from the residue, due to the contact area being less than when applied as a spray dilution. This effect, may have contributed to the lower absorption values observed from residue applications thus far. However, it should be noted, that these values are still relevant to the exposure scenario, as a similar phenomenon would likely occur *in vivo* for a re-entry worker.

6.2 Applicability to the *in vivo* human exposure scenario

Within this document an attempt has been made to relate results back to the re-entry worker exposure scenario. However, it should be noted that the results presented here do not allow firm conclusions to be drawn about *in vivo* human absorption. Although porcine skin is generally considered to be a good model[95-97], inter-species differences can cause differences in absorption when compared to human skin. EFSA do not currently accept studies conducted using pig skin to be used for risk assessment purposes. Current EFSA guidance for *in vitro* absorption studies recommends 8 replicates using human skin from at least 4 donors[10]. With OECD guidance recommending a minimum of 4 replicates from 2 donors[90]. The use of more than one donor is recommended so that the results are representative of the population, and is essential if the aim of the study is to obtain absolute absorption factors for use in risk assessment. The aim of the current study was not to obtain absorption factors, but to validate new methodology and compare exposure conditions, therefore skin from only one

donor was used within each investigation, to reduce inter-individual variability. However, throughout the course of the work, skin from 5 pigs was used, and a retrospective analysis of inter-individual difference is outlined below in section **6.3**.

For firm conclusions to be drawn about residue absorption in re-entry workers, the methodology must be further validated. One method to achieve this could be investigation *in vitro* using human skin. Similar studies to those described here could be performed using human skin from several donors. Further validation could be achieved by investigation *in vivo* using an animal model. The most common animal used rat, this is due to an abundance of data and a well-defined protocol. It is becoming more common to use minipig[135] as the skin more closely models that of human, however, this is not currently accepted by EFSA. Studies investigating dermal absorption of pesticides *in vivo* with human volunteers are deemed un-ethical in the EU. However, *in vivo* studies could be performed using a model compound such as ibuprofen, similar to that described by Belsey et al[21]. A basic study protocol may, for example, apply an ibuprofen solution to a subject's forearm on one visit, and an ibuprofen residue on a second visit. Urine samples and tape-stripping could then be taken to assess differences in absorption between the application types. One limitation could be that the volunteer would be required to remain still for the exposure duration, so as not to dislodge any of the residue from their forearm.

6.3 Retrospective analysis of Inter-Individual Difference

Over the course of the works carried out in this thesis over 280 diffusion cell replicates have been carried out. Experiments that involved application of 30 μL of the spray dilution and transfer of residue from a 40 μg disc were performed frequently as a control. These experiments were repeated 46 times for TXP and 32 times for CLF over the course of 3 years. Over these three years 5 different pig donors were available as a source of skin. Within each set of experiments the donor was kept constant, however, between sets different donors were usually used. Below is retrospective analysis of the effect of inter-individual difference from these control experiments. The protocols were identical, apart from a few minor deviations (e.g., inclusion of Volpo in the receptor solution, which was not expected to influence the results for TXP). The only notable differences for TXP were application volumes; 20 μL for Pig 1, 25 μL for Pig 2 and 30 μL for the other three studies. Residue experiments were generally well matched to the liquid application within each study. Results from 4 different pigs are compiled in Figure 31, full results tables can be found in Appendix 1.

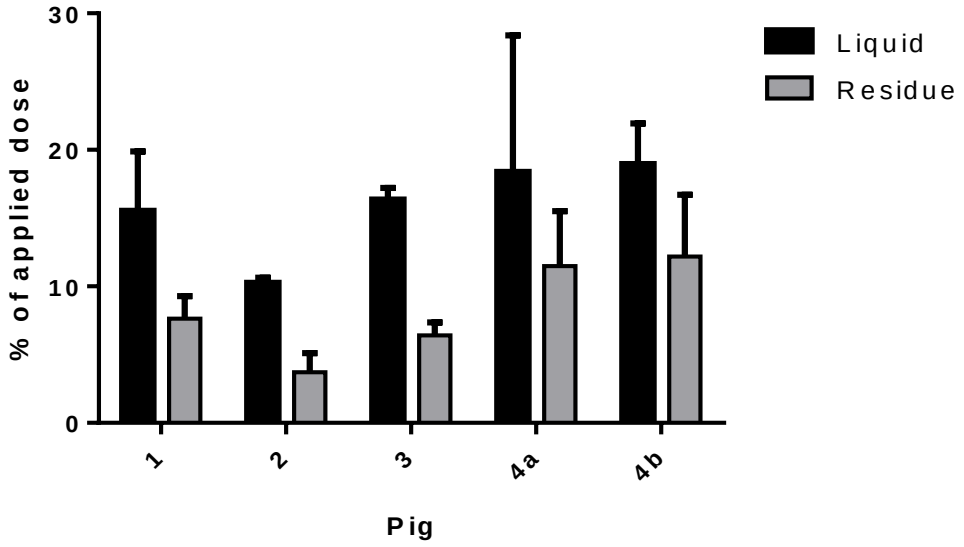

Figure 31 - Retrospective analysis of inter-individual difference in absorption of TXP from EC-A formulation. Results were taken from (1) Paper 1 (n = 5) (2) Paper 2, AI comparison (n = 3 – 7) (3) Paper 3 Loading dose (n = 3-4) (4a) Paper 2 exposure time (n = 3-4) and (4b) Paper 3 formulation (n = 5).

A 2-way analysis of variance revealed a significant difference between pigs ($p < 0.0001$) and found that TXP was absorbed significantly less when applied as a residue than from liquid application across the entire data set ($p < 0.0001$). There was found to be no interaction between the two variables, which shows that although absorption varied between donors, the ratio between liquid and residue remained relatively constant. Pig 2 had the largest difference between application types with TXP from liquid absorbed 2.8 times more. Pig 3 had the smallest difference, with TXP from liquid absorbed 1.7 times more on average. Pig 4 was used for both the exposure time experiments (Pig 4a) and the formulation experiments (Pig 4b), the mean observed % absorption was essentially the same each time. This shows that the differences observed between the data sets is due to inter-individual difference rather than the differences being a result of experiments carried out at different times. The similarity between these two data sets, that were produced more than 6 months apart, also serves to demonstrate the reproducibility of the experimental protocol used.

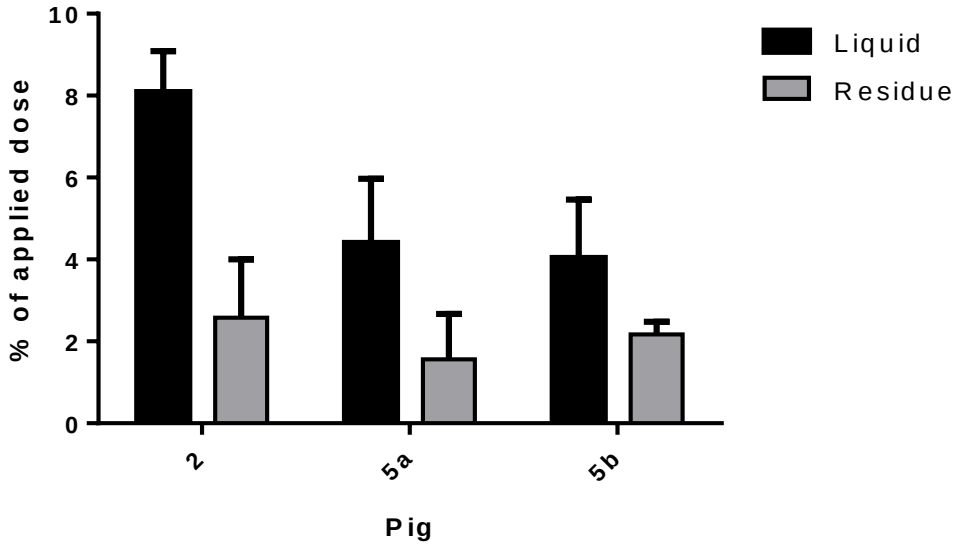

Figure 32 - Retrospective analysis of inter-individual difference in absorption of CLF when applied from EC-A formulation. Results were taken from (2) Paper 2, AI comparison (n = 6), (5a) Paper 3 loading (n = 3-5) and (5b) Paper 3 formulation (n = 6).

CLF experiments were carried out for three separate studies, however, the same donor was used in the studies investigating loading (Pig 5a) and formulation (Pig 5b). Results from the two papers were obtained 3 months apart and again the results were very similar.

A 2-way analysis of variance of CLF results again found a significant difference between pigs and application type. There was also a significant interaction between the two variables ($p < 0.001$), suggesting that the donor may have had an effect upon the difference between liquid and residue. Residue absorption from Pig 1 was 3.1 times less than the liquid compared to only 2.2 times for pig 5 overall and only 1.9 times from results with Pig 5b.

7 CONCLUSIONS

The work carried out in this thesis is the first to quantify dermal absorption of pesticides from dried residues, under non-occlusive conditions. The methodology developed has been proven reproducible over the course of several studies; investigating various AIs, formulations, dose levels and exposure times. In general, these studies have shown that dermal absorption from a dried residue is lower than that from its corresponding spray dilution. However, this was not always observed, absorption of TXP from a WP formulation was not significantly different between the residue and liquid applications. This exception to the general trend suggests that formulation may be an important factor in dermal absorption from dried residues. This demonstrates the need to investigate a larger sample of AI-formulation combinations before general conclusions can be drawn. The findings also highlight the importance of investigating each compound of interest in a relevant formulation if results are to be used for risk assessment.

Additionally, absorption of higher doses of CLF were not significantly different between the two application types, although residue absorption was still lower on average. Fractional absorption from the spray dilution was negatively affected by increasing dose, whereas absorption from the residue remained unaffected. This may be because from a liquid application, an increase in dose did not result in a proportional increase in contact area. Whereas, for the residue, due to lack of a vehicle, it did. This theory, by extension, suggests that the lower residue application may not be covering the entire surface area of the skin. This therefore, could be part of the reason why absorption is less from the residue compared to the spray dilution. It should be noted that these dose levels are towards the higher end of those obtained from the exposure assessment which, with its many built in conservatisms means that these doses are likely higher than a worker would ever be exposed to. Indeed, it is unlikely that a worker would ever be

exposed to a dose level that is flux limited in this way, excess pesticide may not remain on the skin but run off if liquid or fail to adhere if residue.

Investigation of how the duration of exposure to TXP can affect total absorption found that it is important to wash as soon as possible after exposure. When applied as a spray dilution, if decontamination of the skin occurred after more than 2 hours, total TXP absorption after 24 hours was the same as if the skin had not been decontaminated at all. However, when applied as a residue, in general, total absorption decreased as a function of how early decontamination occurred. These findings highlight the importance of regular washing for a re-entry worker to reduce systemic exposure. However, as this study was only carried out with one compound, in one formulation, generalised conclusions should be drawn with caution. This effect may well be influenced by the physicochemical properties of the AI, as well as its formulation.

7.1 Impact and future work

The methodology developed has the potential to be used to carry out diffusion cell experiments for use in regulatory risk assessment. In the case where a product has failed based on a re-entry worker assessment, it may be possible to use this methodology to obtain more realistic fractional absorption values from the specific dried residue in question. In this case, further refinement of the methodology could be possible. For example, if there is a specific foliar surface in question, it may be possible to use this surface to create the residue, thereby making the results obtained more realistic to the scenario. It could even be possible to take leaf samples from an area that has been sprayed and use these to proceed with an experiment. Although this approach would give the most accurate values, as described above, it also adds new uncertainty factors. It would be more difficult to accurately state how much of the pesticide has been applied to the skin. There may also be issues with leaf wax transferring to the skin along

with the AI, although this is arguably more realistic, assuming that the transfer procedure is similar to that which a worker would experience.

Another factor that should be considered, is the presence of moisture in the system. This moisture could arise from many sources including, precipitation, sweat or increased humidity. It is likely that highly water soluble molecules would be affected more by the addition of moisture. An increase in moisture may affect the transfer efficiency of the residue to the skin. Upon being transferred, it may also be absorbed differently to one that is 'drier' and crystalline in nature. If sufficient moisture is present then this may be enough to resolubilise the residue, restoring it to something resembling a spray dilution.

Although the work here has investigated four compounds, this only represents a small sample of pesticide AIs currently in use. In order to fully validate the methodology and to be able to draw general conclusions about residue behaviour, as many products as possible should be investigated. Similarly, although three different formulation and the AI alone have been investigated there are many more types of formulation that have not been investigated, as well as different excipients that exist within these types of formulation.

The doses used in this study were more realistic than those used previously, however, it should be noted that a worker is likely to be exposed to doses lower than those used here. With the aid of radiolabelled compounds, it would be useful to carry out experiments with lower doses. However, this creates issues such as uncertainty of how well spread the dose is, especially from residue application. It is unlikely that a dose level of 0.01μg/cm^2 for example would cover the entire area, this could create artificially low absorption values.

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APPENDIX – FULL RESULTS TABLES

TXP Liquid							
Replicate Number	1	2	3	4	5	**Mean**	**StDev**
Applied (µg)	20	20	20	20	20	**20**	
Recovered in swabs (µg)	12.89	9.15	12.53	9.16	11.16	**10.98**	**1.78**
Total recovery (%)	76.84	68.4	83.15	72.02	73.27	**74.74**	**5.00**
Disposition							
SC tape-strips 1-2 (µg)	0.33	0.64	1.50	1.08	0.78	**0.86**	**0.45**
SC tape-strips 3-15 (µg)	0.18	0.25	0.48	0.4	0.27	**0.32**	**0.12**
SC tape-strips 1-15 (µg)	0.51	0.88	1.98	1.48	1.04	**1.18**	**0.57**
Remaining skin (µg)	0.80	2.13	0.54	1.34	0.61	**1.08**	**0.66**
Receptor phase at 2 hr (µg)	< LOQ	< LOQ	< LOQ	< LOQ	0.39	**0.08**	**0.17**
Receptor phase at 4 hr (µg)	0.34	0.47	0.40	0.58	0.80	**0.52**	**0.18**
Receptor phase at 6 hr (µg)	0.61	0.65	0.67	0.87	0.85	**0.73**	**0.12**
Receptor phase at 8 hr (µg)	0.76	0.90	0.73	1.05	1.09	**0.91**	**0.16**
Receptor phase at 24 hr (µg)	1.18	1.51	1.58	2.42	1.83	**1.71**	**0.47**
Total uptake/absorption (µg)[#]	2.15	3.89	2.60	4.16	2.71	**3.11**	**0.87**
% uptake/absorption of amount applied	10.77	19.45	13.02	20.82	13.57	**15.53**	**4.36**

Table A1 1- Full Results from Paper 1, TXP liquid application

TXP Residue							
Replicate Number	1	2	3	4	5	**Mean**	**StDev**
Applied (µg)	22.99	17.82	26.43	19.94	21.20	**21.68**	**3.26**
Recovered in swabs (µg)	15.58	11.58	13.87	8.57	11.37	**12.19**	**2.67**
Total recovery (%)	88.39	66.21	85.6	51.43	68.84	**72.09**	**13.57**
Disposition							
SC tape-strips 1-2 (µg)	0.56	0.58	0.81	0.42	0.43	**0.56**	**0.16**
SC tape-strips 3-15 (µg)	0.08	0.17	0.35	0.19	0.36	**0.23**	**0.12**
SC tape-strips 1-15 (µg)	0.63	0.74	1.17	0.61	0.79	**0.79**	**0.22**
Remaining skin (µg)	0.43	0.22	0.38	0.20	0.28	**0.30**	**0.10**
Receptor phase at 2 hr (µg)	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	**< LOQ**	**< LOQ**
Receptor phase at 4 hr (µg)	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	**< LOQ**	**< LOQ**
Receptor phase at 6 hr (µg)	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	**< LOQ**	**< LOQ**
Receptor phase at 8 hr (µg)	< LOQ	< LOQ	0.34	< LOQ	< LOQ	**0.07**	**0.15**
Receptor phase at 24 hr (µg)	1.03	0.7	1.72	0.91	1.39	**1.15**	**0.40**
Total uptake/absorption (µg)[#]	1.54	1.09	2.45	1.30	2.03	**1.68**	**0.56**
% uptake/absorption of amount applied	6.69	6.11	9.28	6.52	9.56	**7.63**	**1.65**

Table A1 2- Full results from Paper 1, TXP residue application

TXP Liquid 24 hour exposure							
	1	2	3	4	5	**Mean**	**Stdev**
Applied (µg)	20	20	20	20	20	**20**	
Recovered in swabs (µg)	3.70	8.77	6.95	9.03	7.65	**7.22**	**2.14**
Total recovery (%)	33.83	68.27	56.40	64.59	61.05	**56.83**	**13.59**
Disposition							
SC tape-strips 1-2 (µg)	0.47	0.92	0.74	0.67	0.61	**0.68**	**0.16**
SC tape-strips 3-15 (µg)	0.32	0.47	0.29	0.25	0.45	**0.36**	**0.10**
SC tape-strips 1-15 (µg)	0.80	1.39	1.03	0.92	1.06	**1.04**	**0.22**
Remaining skin (µg)	0.98	0.79	0.90	1.02	0.74	**0.89**	**0.12**
Receptor phase at 2 hr (µg)	< LOQ	< LOQ	< LOQ	0.34	0.28	**0.12**	**0.17**
Receptor phase at 4 hr (µg)	0.45	0.86	0.54	0.37	0.54	**0.55**	**0.19**
Receptor phase at 6 hr (µg)	0.77	1.27	0.73	0.47	0.82	**0.81**	**0.29**
Receptor phase at 8 hr (µg)	0.74	1.31	0.80	0.51	1.17	**0.91**	**0.33**
Receptor phase at 24 hr (µg)	1.29	2.71	2.39	1.94	2.76	**2.22**	**0.61**
Total uptake/absorption (µg)[#]	2.59	3.96	3.59	3.22	3.95	**3.46**	**0.58**
% uptake/absorption of amount applied	12.94	19.80	17.93	16.08	19.77	**17.30**	**2.88**

Table A1 3- Full Results from Paper 1, TXP liquid application, 24-hour exposure.

TXP Residue 24 hour wash						
	1	2	3	4	**Mean**	**Stdev**
Applied (µg)	18.15	23.10	20.52	20.09	**20.47**	**2.03**
Recovered in swabs (µg)	8.97	9.42	9.25	6.85	**8.62**	**1.19**
Total recovery (%)	58.66	71.00	67.83	52.27	**62.44**	**8.56**
Disposition						
SC tape-strips 1-2 (µg)	0.61	1.02	0.85	0.64	**0.78**	**0.19**
SC tape-strips 3-15 (µg)	0.31	0.49	0.38	0.34	**0.38**	**0.08**
SC tape-strips 1-15 (µg)	0.92	1.51	1.22	0.97	**1.16**	**0.27**
Remaining skin (µg)	0.52	0.89	0.90	0.70	**0.75**	**0.18**
Receptor phase at 2 hr (µg)	< LOQ	< LOQ	< LOQ	< LOQ	**< LOQ**	**< LOQ**
Receptor phase at 4 hr (µg)	< LOQ	< LOQ	< LOQ	< LOQ	**< LOQ**	**< LOQ**
Receptor phase at 6 hr (µg)	< LOQ	< LOQ	< LOQ	0.23	**0.06**	**0.11**
Receptor phase at 8 hr (µg)	0.34	0.63	0.66	0.41	**0.51**	**0.16**
Receptor phase at 24 hr (µg)	1.33	2.37	2.20	1.93	**1.96**	**0.46**
Total uptake/absorption (µg)[#]	2.15	3.76	3.47	2.97	**3.09**	**0.70**
% uptake/absorption of amount applied	11.87	16.27	16.92	14.76	**14.95**	**2.25**

Table A1 4 - Full results from Paper 1, TXP residue application, 24-hour exposure

TXP Liquid					
Replicate Number	1	2	3	**Mean**	**StDev**
Applied (µg)	25.00	25.00	25.00	**25.00**	
Recovered in swabs (µg)	12.04	9.23	12.07	**11.12**	**1.63**
Total recovery (%)	71.83	58.13	69.66	**66.54**	**7.36**
Donor wash (µg)	2.21	1.72	1.35	**1.76**	**0.43**
Disposition					
SC tape-strips 1-2 (µg)	1.03	1.03	1.52	**1.20**	**0.28**
SC tape-strips 3-15 (µg)	0.28	0.28	0.44	**0.33**	**0.09**
SC tape-strips 1-15 (µg)	1.31	1.32	1.96	**1.53**	**0.37**
Remaining skin (µg)	0.68	0.89	0.63	**0.73**	**0.14**
Receptor phase at 2 hr (µg)	0.47	0.21	0.29	**0.32**	**0.13**
Receptor phase at 4 hr (µg)	1.02	0.60	0.67	**0.76**	**0.22**
Receptor phase at 6 hr (µg)	1.19	0.83	0.85	**0.96**	**0.20**
Receptor phase at 8 hr (µg)	1.39	0.99	0.99	**1.12**	**0.23**
Receptor phase at 24 hr (µg)	1.71	1.38	1.41	**1.50**	**0.18**
Total uptake/absorption (µg)[#]	2.67	2.55	2.47	**2.56**	**0.10**
% uptake/absorption of amount applied	10.69	10.18	9.89	**10.25**	**0.40**

Table A1 5- Full Results from Paper 2, TXP liquid

TXP Residue									
Replicate Number	1	2	3	4	5	6	7	**Mean**	**StDev**
Applied (µg)	23.68	15.28	17.86	27.04	24.33	23.64	25.34	**22.45**	**4.25**
Recovered in swabs (µg)	15.82	8.62	10.20	11.44	10.47	9.15	11.68	**11.06**	**2.38**
Total recovery (%)	83.80	87.02	83.89	65.51	69.13	70.37	70.98	**75.81**	**8.74**
Donor wash (µg)	0.21	0.20	0.44	0.61	0.35	0.29	0.49	**0.37**	**0.15**
Disposition									
SC tape-strips 1-2 (µg)	0.46	0.54	0.27	0.63	0.52	0.99	0.32	**0.53**	**0.24**
SC tape-strips 3-15 (µg)	0.12	0.14	0.11	0.11	0.07	0.41	0.23	**0.17**	**0.12**
SC tape-strips 1-15 (µg)	0.58	0.68	0.37	0.74	0.60	1.40	0.56	**0.70**	**0.33**
Remaining skin (µg)	0.30	0.31	0.21	0.45	0.23	0.62	0.52	**0.38**	**0.16**
Receptor phase at 2 hr (µg)	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	**< LOQ**	**< LOQ**
Receptor phase at 4 hr (µg)	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	**< LOQ**	**< LOQ**
Receptor phase at 6 hr (µg)	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	**< LOQ**	**< LOQ**
Receptor phase at 8 hr (µg)	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	**< LOQ**	**< LOQ**
Receptor phase at 24 hr (µg)	0.29	0.27	0.23	< LOQ	0.33	0.33	0.49	**0.27**	**0.15**
Total uptake/absorption (µg)[#]	0.70	0.72	0.54	0.56	0.64	1.36	1.25	**0.82**	**0.34**
% uptake/absorption of amount applied	2.97	4.72	3.01	2.07	2.61	5.75	4.92	**3.72**	**1.39**

Table A1 6 - Full Results from Paper 2, TXP Residue

CLF Liquid								
Replicate Number	1	2	3	4	5	6	**Mean**	**StDev**
Applied (µg)	30	30	30	30	30	30	**30**	
Recovered in swabs (µg)	19.56	17.29	23.97	20.04	23.29	22.37	**21.09**	**2.56**
Total recovery (%)	84.15	73.90	99.27	84.56	98.61	91.79	**88.72**	**9.76**
Donor wash (µg)	0.81	0.68	0.89	1.10	0.85	0.84	**0.86**	**0.14**
Disposition								
SC tape-strips 1-2 (µg)	2.79	2.06	2.58	1.75	2.57	1.69	**2.24**	**0.47**
SC tape-strips 3-15 (µg)	0.62	0.73	0.71	0.86	0.93	0.88	**0.79**	**0.12**
SC tape-strips 1-15 (µg)	3.41	2.80	3.28	2.60	3.50	2.57	**3.03**	**0.42**
Remaining skin (µg)	1.47	1.41	1.64	1.63	1.94	1.76	**1.64**	**0.19**
Receptor phase at 24 hr (µg)	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	**< LOQ**	**< LOQ**
Total uptake/absorption (µg)*	2.08	2.14	2.35	2.49	2.88	2.64	**2.43**	**0.30**
% uptake/absorption of amount applied	6.95	7.13	7.83	8.28	9.59	8.79	**8.09**	**1.00**

Table A1 7- Full Results from Paper 2, CLF liquid application.

CLF Residue								
Replicate Number	1	2	3	4	5	6	**Mean**	**StDev**
Applied (µg)	31.20	31.27	31.58	28.70	31.96	25.12	**29.97**	**2.64**
Recovered in swabs (µg)	29.08	28.49	25.87	20.98	20.99	17.03	**23.74**	**4.81**
Total recovery (%)	102.37	101.07	95.56	83.78	80.80	82.43	**91.00**	**9.81**
Donor wash (µg)	0.81	0.91	0.87	0.27	0.53	0.16	**0.59**	**0.32**
Disposition								
SC tape-strips 1-2 (µg)	1.44	1.48	1.46	0.63	1.89	0.58	**1.25**	**0.52**
SC tape-strips 3-15 (µg)	0.35	0.17	0.30	0.14	0.46	0.11	**0.25**	**0.14**
SC tape-strips 1-15 (µg)	1.79	1.65	1.76	0.77	2.35	0.69	**1.50**	**0.65**
Remaining skin (µg)	0.47	0.65	1.31	0.19	0.41	0.20	**0.54**	**0.41**
Receptor phase at 24 hr (µg)	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	**< LOQ**	**< LOQ**
Total uptake/absorption (µg)*	0.82	0.82	1.61	0.33	0.87	0.32	**0.79**	**0.47**
% uptake/absorption of amount applied	2.63	2.61	5.09	1.14	2.72	1.27	**2.58**	**1.42**

Table A1 8- Full Results from Paper 2, CLF Residue application

DFZ Liquid								
Replicate Number	1	2	3	4	5	6	**Mean**	**StDev**
Applied (µg)	30	30	30	30	30	30	**30**	
Recovered in swabs (µg)	24.85	23.45	23.78	24.80	21.66	27.33	**24.31**	**1.88**
Total recovery (%)	98.06	96.12	98.90	98.70	87.44	102.43	**96.94**	**5.09**
Donor wash (µg)	0.76	0.58	1.04	0.35	0.95	0.62	**0.72**	**0.25**
Disposition								
SC tape-strips 1-2 (µg)	0.73	1.95	2.37	1.66	1.54	1.07	**1.55**	**0.59**
SC tape-strips 3-15 (µg)	0.84	0.44	0.76	0.65	0.43	0.29	**0.57**	**0.21**
SC tape-strips 1-15 (µg)	1.57	2.39	3.12	2.32	1.97	1.36	**2.12**	**0.64**
Remaining skin (µg)	2.25	2.41	1.73	2.15	1.65	1.42	**1.93**	**0.39**
Receptor phase at 24 hr (µg)	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	**< LOQ**	**< LOQ**
Total uptake/absorption (µg)[#]	3.09	2.85	2.48	2.80	2.08	1.71	**2.50**	**0.52**
% uptake/absorption of amount applied	10.29	9.51	8.28	9.32	6.95	5.70	**8.34**	**1.74**

Table A1 9- Full Results from Paper 2, DFZ liquid application.

DFZ Residue													
Replicate Number	1	2	3	4	5	6	7	8	9	10	11	**Mean**	**StDev**
Applied (µg)	27.76	29.70	27.38	33.30	36.23	29.72	30.38	26.38	25.96	24.13	28.15	**29.01**	**3.44**
Recovered in swabs (µg)	18.44	21.89	22.59	26.72	29.40	23.69	22.30	18.96	20.45	19.38	23.11	**22.45**	**3.32**
Total recovery (%)	81.13	80.63	92.71	89.59	91.67	91.72	86.27	83.14	88.02	90.81	90.19	**87.81**	**4.39**
Donor wash (µg)	0.60	0.61	0.38	0.61	0.76	0.51	0.76	0.35	0.37	0.34	0.29	**0.51**	**0.17**
Disposition													
SC tape-strips 1-2 (µg)	1.71	0.65	1.31	1.37	1.22	1.31	1.43	1.16	0.97	0.99	0.60	**1.16**	**0.33**
SC tape-strips 3-15 (µg)	0.66	0.25	0.28	0.33	0.56	0.42	0.29	0.20	0.28	0.26	0.15	**0.34**	**0.15**
SC tape-strips 1-15 (µg)	2.36	0.90	1.59	1.71	1.78	1.73	1.72	1.36	1.25	1.26	0.75	**1.49**	**0.45**
Remaining skin (µg)	1.12	0.55	0.82	0.81	1.28	1.33	1.42	1.27	0.78	0.93	1.23	**1.05**	**0.28**
Receptor phase at 24 hr (µg)	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	**< LOQ**	**< LOQ**
Total uptake/absorption (µg)[#]	1.78	0.80	1.10	1.14	1.83	1.75	1.71	1.47	1.06	1.20	1.38	**1.38**	**0.35**
% uptake/absorption of amount applied	6.41	2.69	4.02	3.43	5.06	5.89	5.64	5.58	4.08	4.96	4.90	**4.79**	**1.13**

Table A1 10 - Full Results from Paper 2, DFZ residue application

PPZ Liquid								
Replicate Number	1	2	3	4	5	6	**Mean**	**StDev**
Applied (µg)	25	25	25	25	25	25	**25**	
Recovered in swabs (µg)	16.04	13.80	15.22	13.43	8.89	8.16	**12.59**	**3.30**
Total recovery (%)	105.12	80.84	100.27	84.98	76.25	63.55	**85.17**	**15.44**
Donor wash (µg)	1.56	0.71	1.46	1.24	1.86	1.74	**1.43**	**0.41**
Disposition								
SC tape-strips 1-2 (µg)	5.04	1.62	3.09	2.10	2.96	2.16	**2.83**	**1.22**
SC tape-strips 3-15 (µg)	1.92	1.90	1.77	1.82	2.27	1.49	**1.86**	**0.25**
SC tape-strips 1-15 (µg)	6.96	3.52	4.86	3.92	5.23	3.65	**4.69**	**1.31**
Remaining skin (µg)	1.72	2.18	3.52	2.65	3.08	2.34	**2.58**	**0.65**
Receptor phase at 8 hr (µg)	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	**< LOQ**	**< LOQ**
Receptor phase at 24 hr (µg)	0.54	0.45	1.07	0.82	1.05	0.57	**0.75**	**0.27**
Total uptake/absorption (µg)[#]	4.18	4.53	6.35	5.30	6.40	4.40	**5.19**	**0.99**
% uptake/absorption of amount applied	16.73	18.13	25.41	21.18	25.58	17.59	**20.77**	**3.96**

Table A1 11 - Full Results from Paper 2, PPZ liquid application.

PPZ Residue								
Replicate Number	1	2	3	4	5	6	**Mean**	**StDev**
Applied (µg)	24.10	22.53	26.14	24.93	23.67	25.29	**24.44**	**1.28**
Recovered in swabs (µg)	20.33	17.29	21.79	19.45	20.85	20.00	**19.95**	**1.53**
Total recovery (%)	102.78	97.68	100.45	99.34	102.75	101.64	**100.77**	**2.02**
Donor wash (µg)	0.66	0.59	0.84	0.86	0.66	0.85	**0.74**	**0.12**
Disposition								
SC tape-strips 1-2 (µg)	1.91	2.14	1.69	2.16	1.15	2.16	**1.87**	**0.40**
SC tape-strips 3-15 (µg)	1.28	1.21	1.37	1.25	1.25	1.65	**1.33**	**0.16**
SC tape-strips 1-15 (µg)	3.19	3.34	3.06	3.40	2.40	3.81	**3.20**	**0.47**
Remaining skin (µg)	0.59	0.79	0.57	1.05	0.42	1.06	**0.75**	**0.27**
Receptor phase at 24 hr (µg)	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	**< LOQ**	**< LOQ**
Total uptake/absorption (µg)[#]	1.87	1.99	1.94	2.29	1.67	2.71	**2.08**	**0.37**
% uptake/absorption of amount applied	7.75	8.85	7.43	9.21	7.04	10.69	**8.50**	**1.36**

Table A1 12- Full Results from Paper 2, PPZ residue application.

TXP Liquid 0.5 hr wash					
Replicate Number	1	2	3	**Mean**	**StDev**
Applied (µg)	30	30	30	**30**	
Recovered in swabs (µg)	21.87	19.32	20.60	**20.59**	**1.28**
Total recovery (%)	82.95	74.45	79.50	**78.96**	**4.28**
Donor wash (µg)	0.84	0.42	0.63	**0.63**	**0.21**
Disposition					
SC tape-strips 1-2 (µg)	0.41	0.75	0.80	**0.65**	**0.21**
SC tape-strips 3-15 (µg)	0.07	0.12	0.10	**0.10**	**0.02**
SC tape-strips 1-15 (µg)	0.48	0.87	0.90	**0.75**	**0.23**
Remaining skin (µg)	0.80	0.47	0.60	**0.62**	**0.17**
Receptor phase at 2 hr (µg)	0.58	0.67	0.64	**0.63**	**0.04**
Receptor phase at 3 hr (µg)	0.71	0.98	0.76	**0.82**	**0.15**
Receptor phase at 4 hr (µg)	0.74	1.11	0.86	**0.90**	**0.19**
Receptor phase at 5 hr (µg)	0.73	0.92	0.87	**0.84**	**0.10**
Receptor phase at 6 hr (µg)	0.77	0.96	0.92	**0.89**	**0.10**
Receptor phase at 7 hr (µg)	0.73	1.04	0.95	**0.91**	**0.16**
Receptor phase at 8 hr (µg)	0.73	0.93	0.94	**0.86**	**0.12**
Receptor phase at 24 hr (µg)	0.88	1.27	1.13	**1.10**	**0.20**
Total uptake/absorption (µg)[#]	1.76	1.86	1.83	**1.81**	**0.05**
% uptake/absorption of amount applied	5.86	6.19	6.08	**6.04**	**0.17**

Table A1 13- Full Results from Paper 2, TXP 0.5-hr exposure liquid application

TXP Residue 0.5 hr wash					
Replicate Number	1	2	3	**Mean**	**StDev**
Applied (µg)	29.48	28.44	28.47	**28.79**	**0.59**
Recovered in swabs (µg)	17.20	18.29	19.51	**18.33**	**1.16**
Total recovery (%)	72.34	79.08	81.89	**77.77**	**4.91**
Donor wash (µg)	0.85	0.93	0.89	**0.89**	**0.04**
Disposition					
SC tape-strips 1-2 (µg)	0.07	0.53	0.10	**0.23**	**0.26**
SC tape-strips 3-15 (µg)	< LOQ	< LOQ	0.06	**0.02**	**0.03**
SC tape-strips 1-15 (µg)	0.07	0.53	0.16	**0.25**	**0.24**
Remaining skin (µg)	< LOQ	0.32	0.28	**0.20**	**0.17**
Receptor phase at 2 hr (µg)	0.18	< LOQ	< LOQ	**0.06**	**0.10**
Receptor phase at 3 hr (µg)	0.28	< LOQ	< LOQ	**0.09**	**0.16**
Receptor phase at 4 hr (µg)	0.26	< LOQ	< LOQ	**0.09**	**0.15**
Receptor phase at 5 hr (µg)	0.27	< LOQ	< LOQ	**0.09**	**0.16**
Receptor phase at 6 hr (µg)	0.29	< LOQ	0.19	**0.16**	**0.15**
Receptor phase at 7 hr (µg)	0.27	< LOQ	0.20	**0.15**	**0.14**
Receptor phase at 8 hr (µg)	0.30	< LOQ	0.25	**0.18**	**0.16**
Receptor phase at 24 hr (µg)	0.29	< LOQ	0.37	**0.22**	**0.20**
Total uptake/absorption (µg)[#]	0.29	0.32	0.71	**0.44**	**0.24**
% uptake/absorption of amount applied	0.97	1.12	2.51	**1.53**	**0.85**

Table A1 14 - Full Results from Paper 2, TXP 0.5-hr exposure Residue application

TXP Liquid 1 hour wash					
Replicate Number	1	2	3	**Mean**	**StDev**
Applied (µg)	30	30	30	**30**	
Recovered in swabs (µg)	18.28	24.60	25.77	**22.88**	**4.03**
Total recovery (%)	75.14	98.28	99.56	**91.00**	**13.75**
Donor wash (µg)	1.18	0.78	0.37	**0.78**	**0.41**
Disposition					
SC tape-strips 1-2 (µg)	0.31	0.55	0.41	**0.42**	**0.12**
SC tape-strips 3-15 (µg)	0.05	0.29	0.23	**0.19**	**0.12**
SC tape-strips 1-15 (µg)	0.36	0.84	0.64	**0.61**	**0.24**
Remaining skin (µg)	1.19	0.56	0.57	**0.77**	**0.36**
Receptor phase at 2 hr (µg)	1.16	2.06	1.76	**1.66**	**0.46**
Receptor phase at 3 hr (µg)	1.44	2.38	2.12	**1.98**	**0.49**
Receptor phase at 4 hr (µg)	1.53	2.56	2.24	**2.11**	**0.53**
Receptor phase at 5 hr (µg)	1.60	2.70	2.22	**2.17**	**0.55**
Receptor phase at 6 hr (µg)	1.67	2.78	2.44	**2.30**	**0.57**
Receptor phase at 7 hr (µg)	1.67	2.77	2.47	**2.30**	**0.57**
Receptor phase at 8 hr (µg)	1.57	2.76	2.54	**2.29**	**0.63**
Receptor phase at 24 hr (µg)	1.53	2.71	2.52	**2.25**	**0.63**
Total uptake/absorption (µg)[#]	2.78	3.56	3.32	**3.22**	**0.40**
% uptake/absorption of amount applied	9.26	11.86	11.06	**10.73**	**1.33**

Table A1 15 - Full Results from Paper 2, TXP 1-hr exposure Liquid application

TXP Residue 1 hour wash					
Replicate Number	1	2	3	**Mean**	**StDev**
Applied (µg)	23.79	28.32	28.40	**26.84**	**2.64**
Recovered in swabs (µg)	15.44	18.38	19.21	**17.67**	**1.98**
Total recovery (%)	86.06	83.43	82.64	**84.04**	**1.79**
Donor wash (µg)	1.30	0.82	0.35	**0.82**	**0.48**
Disposition					
SC tape-strips 1-2 (µg)	0.30	0.50	0.32	**0.37**	**0.11**
SC tape-strips 3-15 (µg)	0.02	0.22	0.23	**0.16**	**0.12**
SC tape-strips 1-15 (µg)	0.32	0.72	0.55	**0.53**	**0.20**
Remaining skin (µg)	0.14	0.08	0.17	**0.13**	**0.05**
Receptor phase at 2 hr (µg)	0.89	1.27	0.93	**1.03**	**0.21**
Receptor phase at 3 hr (µg)	0.99	1.37	1.00	**1.12**	**0.22**
Receptor phase at 4 hr (µg)	1.06	1.48	1.09	**1.21**	**0.23**
Receptor phase at 5 hr (µg)	1.10	1.50	1.12	**1.24**	**0.22**
Receptor phase at 6 hr (µg)	1.08	1.64	1.16	**1.29**	**0.30**
Receptor phase at 7 hr (µg)	1.04	1.56	1.18	**1.26**	**0.27**
Receptor phase at 8 hr (µg)	1.00	1.57	1.19	**1.25**	**0.29**
Receptor phase at 24 hr (µg)	1.02	1.69	1.18	**1.30**	**0.35**
Total uptake/absorption (µg)[#]	1.18	1.99	1.58	**1.58**	**0.40**
% uptake/absorption of amount applied	4.96	7.03	5.57	**5.85**	**1.06**

Table A1 16 - Full Results from Paper 2, TXP 1-hr exposure Residue application

TXP Liquid 2 hour wash									
Replicate Number	1	2	3	4	5	6	7	**Mean**	**StDev**
Applied (µg)	30	30	30	30	30	30	30	**30.00**	
Recovered in swabs (µg)	22.33	22.04	18.82	17.84	18.48	17.33	18.23	**19.29**	**2.03**
Total recovery (%)	88.28	85.91	86.39	89.27	96.29	85.68	88.28	**88.59**	**3.66**
Donor wash (µg)	0.47	0.80	0.66	0.35	0.54	0.47	0.43	**0.53**	**0.15**
Disposition									
SC tape-strips 1-2 (µg)	0.72	1.14	0.46	0.62	0.46	0.78	0.20	**0.63**	**0.30**
SC tape-strips 3-15 (µg)	0.22	0.13	0.15	0.44	0.23	0.35	0.04	**0.22**	**0.14**
SC tape-strips 1-15 (µg)	0.94	1.27	0.61	1.06	0.69	1.13	0.24	**0.85**	**0.35**
Remaining skin (µg)	0.98	0.37	2.17	1.39	1.32	1.60	2.24	**1.44**	**0.65**
Receptor phase at 2 hr (µg)	1.07	0.67	1.35	2.48	3.50	1.70	3.24	**2.00**	**1.09**
Receptor phase at 3 hr (µg)	1.60	1.02	2.02	3.56	5.00	2.56	4.22	**2.85**	**1.45**
Receptor phase at 4 hr (µg)	1.89	1.37	2.49	4.04	5.71	2.97	4.62	**3.30**	**1.56**
Receptor phase at 5 hr (µg)	2.00	1.39	2.58	4.31	5.95	3.25	4.77	**3.46**	**1.63**
Receptor phase at 6 hr (µg)	1.94	1.47	2.75	4.72	6.30	3.65	5.07	**3.70**	**1.76**
Receptor phase at 7 hr (µg)	2.01	1.51	2.98	4.77	6.69	3.82	5.18	**3.85**	**1.84**
Receptor phase at 8 hr (µg)	1.95	1.39	3.07	4.86	6.69	4.00	5.26	**3.89**	**1.89**
Receptor phase at 24 hr (µg)	1.76	1.29	3.66	6.16	7.86	5.18	5.35	**4.47**	**2.37**
Total uptake/absorption (µg)[#]	2.96	1.79	5.98	7.99	9.41	7.12	7.63	**6.13**	**2.78**
% uptake/absorption of amount applied	9.88	5.97	19.93	26.63	31.37	23.73	25.42	**20.42**	**9.26**

Table A1 17 - Full Results from Paper 2, TXP 2-hr exposure Liquid application.

TXP Residue 2 hour wash									
Replicate Number	1	2	3	4	5	6	7	**Mean**	**StDev**
Applied (µg)	31.99	31.09	26.37	26.60	25.50	29.04	27.67	**28.32**	**2.48**
Recovered in swabs (µg)	17.85	19.03	18.54	17.15	16.99	18.46	19.19	**18.18**	**0.87**
Total recovery (%)	75.51	81.66	88.89	82.39	86.05	79.13	84.63	**82.61**	**4.45**
Donor wash (µg)	0.81	0.61	1.00	0.96	0.47	1.17	0.21	**0.75**	**0.34**
Disposition									
SC tape-strips 1-2 (µg)	1.33	0.69	0.50	0.34	0.42	0.27	0.35	**0.56**	**0.37**
SC tape-strips 3-15 (µg)	0.33	0.25	0.12	0.07	0.05	0.20	0.11	**0.16**	**0.10**
SC tape-strips 1-15 (µg)	1.66	0.94	0.62	0.41	0.47	0.47	0.46	**0.72**	**0.45**
Remaining skin (µg)	0.72	0.84	0.10	< LOQ	0.25	0.19	0.43	**0.36**	**0.32**
Receptor phase at 2 hr (µg)	0.76	1.92	0.94	0.35	1.02	< LOQ	0.61	**0.80**	**0.61**
Receptor phase at 3 hr (µg)	1.15	2.44	1.18	0.59	1.33	0.25	0.80	**1.11**	**0.70**
Receptor phase at 4 hr (µg)	1.24	2.61	1.31	0.64	1.43	0.30	1.00	**1.22**	**0.73**
Receptor phase at 5 hr (µg)	1.29	2.67	1.44	0.73	1.52	0.38	1.04	**1.29**	**0.73**
Receptor phase at 6 hr (µg)	1.30	2.82	1.47	0.77	1.63	0.41	1.11	**1.36**	**0.77**
Receptor phase at 7 hr (µg)	1.35	2.79	1.49	0.73	1.71	0.32	1.14	**1.36**	**0.79**
Receptor phase at 8 hr (µg)	1.29	2.62	1.56	0.75	1.68	0.51	1.19	**1.37**	**0.69**
Receptor phase at 24 hr (µg)	1.15	2.32	1.66	1.02	1.73	0.39	1.23	**1.36**	**0.62**
Total uptake/absorption (µg)[#]	2.20	3.42	1.88	1.09	2.03	0.78	1.77	**1.88**	**0.85**
% uptake/absorption of amount applied	6.89	11.01	7.14	4.12	7.95	2.70	6.40	**6.60**	**2.68**

Table A1 18 - Full Results from Paper 2, TXP 2-hr exposure residue application.

TXP Liquid 4 hour wash									
Replicate Number	1	2	3	4	5	6	7	**Mean**	**StDev**
Applied (µg)	30	30	30	30	30	30	30	**30.00**	
Recovered in swabs (µg)	21.86	22.25	20.36	14.50	13.16	16.66	14.32	**17.59**	**3.84**
Total recovery (%)	86.15	86.14	90.86	76.75	75.76	89.07	77.24	**83.14**	**6.36**
Donor wash (µg)	0.55	0.74	0.74	0.48	0.58	0.70	0.86	**0.66**	**0.13**
Disposition									
SC tape-strips 1-2 (µg)	0.91	0.79	0.76	0.60	0.45	0.42	0.66	**0.66**	**0.18**
SC tape-strips 3-15 (µg)	0.23	0.15	0.25	0.44	0.25	0.26	0.43	**0.29**	**0.11**
SC tape-strips 1-15 (µg)	1.14	0.94	1.01	1.04	0.69	0.68	1.10	**0.94**	**0.18**
Remaining skin (µg)	0.78	0.38	1.13	1.33	1.91	1.26	0.92	**1.10**	**0.48**
Receptor phase at 2 hr (µg)	0.96	0.85	1.55	2.57	3.24	3.18	2.35	**2.10**	**0.99**
Receptor phase at 3 hr (µg)	1.28	1.31	2.16	3.31	4.09	4.26	3.30	**2.82**	**1.24**
Receptor phase at 4 hr (µg)	1.46	1.60	2.46	3.87	4.62	5.39	3.96	**3.34**	**1.52**
Receptor phase at 5 hr (µg)	1.54	1.67	2.85	4.35	4.99	5.80	4.47	**3.67**	**1.66**
Receptor phase at 6 hr (µg)	1.63	1.85	3.07	4.76	5.43	6.53	4.78	**4.01**	**1.86**
Receptor phase at 7 hr (µg)	1.59	1.87	3.12	5.01	5.79	6.75	5.11	**4.18**	**1.99**
Receptor phase at 8 hr (µg)	1.56	1.83	3.29	4.96	5.69	6.88	5.13	**4.19**	**2.01**
Receptor phase at 24 hr (µg)	1.52	1.53	4.02	5.68	6.38	7.42	5.97	**4.65**	**2.36**
Total uptake/absorption (µg)[#]	2.53	2.06	5.40	7.45	8.54	8.95	7.32	**6.03**	**2.79**
% uptake/absorption of amount applied	8.43	6.88	17.99	24.82	28.46	29.82	24.41	**20.12**	**9.32**

Table A1 19 - Full Results from Paper 2, TXP 4-hr exposure liquid application.

TXP Residue 4 hour wash								
Replicate Number	1	2	3	4	5	6	**Mean**	**StDev**
Applied (µg)	23.99	32.86	28.68	25.80	27.38	28.90	**27.93**	**3.04**
Recovered in swabs (µg)	18.01	15.91	14.40	12.54	17.49	17.15	**15.92**	**2.10**
Total recovery (%)	90.64	72.20	76.57	82.16	79.45	76.06	**79.51**	**6.40**
Donor wash (µg)	0.79	0.72	0.44	0.30	0.47	0.98	**0.62**	**0.26**
Disposition								
SC tape-strips 1-2 (µg)	0.30	1.35	0.72	0.50	0.25	0.30	**0.57**	**0.42**
SC tape-strips 3-15 (µg)	0.06	0.36	0.16	0.07	0.03	0.08	**0.13**	**0.12**
SC tape-strips 1-15 (µg)	0.36	1.71	0.88	0.57	0.28	0.38	**0.70**	**0.54**
Remaining skin (µg)	0.28	1.14	0.23	0.24	0.55	0.37	**0.47**	**0.35**
Receptor phase at 2 hr (µg)	0.59	1.38	1.11	2.98	< LOQ	< LOQ	**1.01**	**1.12**
Receptor phase at 3 hr (µg)	0.80	1.83	1.64	3.54	< LOQ	< LOQ	**1.30**	**1.35**
Receptor phase at 4 hr (µg)	0.94	2.11	2.13	3.79	0.26	0.27	**1.58**	**1.37**
Receptor phase at 5 hr (µg)	0.96	2.25	2.70	4.12	0.32	0.29	**1.77**	**1.52**
Receptor phase at 6 hr (µg)	1.02	2.55	2.90	4.30	0.39	0.41	**1.93**	**1.58**
Receptor phase at 7 hr (µg)	1.00	2.51	2.96	4.32	0.45	0.50	**1.95**	**1.56**
Receptor phase at 8 hr (µg)	0.98	2.66	3.07	4.55	0.47	0.51	**2.04**	**1.65**
Receptor phase at 24 hr (µg)	0.80	2.25	3.35	5.01	0.38	0.45	**2.04**	**1.87**
Total uptake/absorption (µg)[#]	1.14	3.75	3.74	5.33	0.95	0.89	**2.63**	**1.89**
% uptake/absorption of amount applied	4.74	11.42	13.06	20.66	3.48	3.09	**9.41**	**6.94**

Table A1 20 - Full Results from Paper 2, TXP 4-hr exposure residue application.

TXP Liquid 8 hour wash					
Replicate Number	1	2	3	**Mean**	**StDev**
Applied (µg)	30	30	30	**30**	
Recovered in swabs (µg)	21.66	14.73	17.86	**18.08**	**3.47**
Total recovery (%)	85.36	79.88	83.22	**82.82**	**2.76**
Donor wash (µg)	0.31	0.56	0.73	**0.53**	**0.21**
Disposition					
SC tape-strips 1-2 (µg)	1.37	0.48	0.29	**0.71**	**0.57**
SC tape-strips 3-15 (µg)	0.35	0.16	0.02	**0.18**	**0.17**
SC tape-strips 1-15 (µg)	1.71	0.65	0.30	**0.89**	**0.74**
Remaining skin (µg)	0.42	1.84	1.69	**1.32**	**0.78**
Receptor phase at 2 hr (µg)	0.78	1.60	< LOQ	**0.79**	**0.80**
Receptor phase at 3 hr (µg)	1.09	2.46	< LOQ	**1.18**	**1.23**
Receptor phase at 4 hr (µg)	1.21	3.18	0.31	**1.57**	**1.47**
Receptor phase at 5 hr (µg)	1.42	3.65	0.44	**1.84**	**1.65**
Receptor phase at 6 hr (µg)	1.57	4.17	0.73	**2.16**	**1.80**
Receptor phase at 7 hr (µg)	1.46	4.70	0.88	**2.35**	**2.06**
Receptor phase at 8 hr (µg)	1.44	4.97	1.15	**2.52**	**2.12**
Receptor phase at 24 hr (µg)	1.51	6.18	4.38	**4.02**	**2.36**
Total uptake/absorption (µg)[#]	2.28	8.19	6.09	**5.52**	**3.00**
% uptake/absorption of amount applied	7.59	27.29	20.30	**18.39**	**9.99**

Table A1 21 - Full Results from Paper 2, TXP 8-hr exposure liquid

TXP Residue 8 hour wash								
Replicate Number	1	2	3	4	5	6	**Mean**	**StDev**
Applied (µg)	27.46	28.62	25.62	29.01	26.02	28.60	**27.56**	**1.45**
Recovered in swabs (µg)	12.63	15.60	23.63	18.85	16.37	17.90	**17.50**	**3.69**
Total recovery (%)	76.05	79.02	105.90	88.69	81.32	79.75	**85.12**	**11.02**
Donor wash (µg)	0.87	0.96	< LOQ	0.33	0.12	0.17	**0.41**	**0.41**
Disposition								
SC tape-strips 1-2 (µg)	0.56	0.73	0.99	0.62	0.40	0.45	**0.63**	**0.21**
SC tape-strips 3-15 (µg)	0.15	0.23	0.45	0.31	0.17	0.12	**0.24**	**0.13**
SC tape-strips 1-15 (µg)	0.71	0.96	1.45	0.93	0.57	0.57	**0.87**	**0.33**
Remaining skin (µg)	0.28	0.40	0.89	0.31	0.54	0.63	**0.51**	**0.23**
Receptor phase at 2 hr (µg)	1.51	0.59	0.65	2.11	< LOQ	< LOQ	**0.81**	**0.84**
Receptor phase at 3 hr (µg)	1.88	0.80	0.88	2.63	< LOQ	0.26	**1.07**	**1.00**
Receptor phase at 4 hr (µg)	2.05	0.98	1.08	2.84	0.28	0.56	**1.30**	**0.97**
Receptor phase at 5 hr (µg)	2.26	1.08	1.18	3.08	0.37	0.62	**1.43**	**1.03**
Receptor phase at 6 hr (µg)	2.39	1.23	1.30	3.51	0.42	0.65	**1.58**	**1.17**
Receptor phase at 7 hr (µg)	2.55	1.24	1.30	3.55	0.48	0.80	**1.65**	**1.17**
Receptor phase at 8 hr (µg)	2.75	1.50	1.39	3.67	0.59	0.80	**1.79**	**1.19**
Receptor phase at 24 hr (µg)	3.39	2.31	2.01	4.39	1.07	1.40	**2.43**	**1.26**
Total uptake/absorption (µg)[#]	3.82	2.94	3.36	5.02	1.77	2.14	**3.18**	**1.18**
% uptake/absorption of amount applied	13.89	10.28	13.12	17.30	6.82	7.50	**11.48**	**4.04**

Table A1 22 - Full Results from Paper 2, TXP 8-hr exposure residue

TXP Liquid 24 hour wash					
Replicate Number	1	2	3	**Mean**	**StDev**
Applied (µg)	30	30	30	**30**	
Recovered in swabs (µg)	19.14	17.51	19.79	**18.81**	**1.17**
Total recovery (%)	87.00	83.06	88.06	**86.04**	**2.63**
Donor wash (µg)	0.47	0.38	0.16	**0.34**	**0.16**
Disposition					
SC tape-strips 1-2 (µg)	0.23	0.71	0.24	**0.39**	**0.27**
SC tape-strips 3-15 (µg)	0.21	0.28	0.23	**0.24**	**0.04**
SC tape-strips 1-15 (µg)	0.44	0.99	0.47	**0.63**	**0.31**
Remaining skin (µg)	0.94	0.59	0.63	**0.72**	**0.19**
Receptor phase at 2 hr (µg)	1.86	2.01	1.65	**1.84**	**0.18**
Receptor phase at 3 hr (µg)	2.40	2.43	2.09	**2.31**	**0.19**
Receptor phase at 4 hr (µg)	2.69	2.75	2.55	**2.67**	**0.10**
Receptor phase at 5 hr (µg)	2.96	3.04	2.88	**2.96**	**0.08**
Receptor phase at 6 hr (µg)	3.21	3.33	3.17	**3.24**	**0.08**
Receptor phase at 7 hr (µg)	3.33	3.35	3.37	**3.35**	**0.02**
Receptor phase at 8 hr (µg)	3.43	3.62	3.49	**3.51**	**0.10**
Receptor phase at 24 hr (µg)	5.11	5.44	5.36	**5.30**	**0.17**
Total uptake/absorption (µg)[#]	6.26	6.32	6.22	**6.27**	**0.05**
% uptake/absorption of amount applied	20.87	21.07	20.74	**20.89**	**0.17**

Table A1 23- Full Results from Paper 2, TXP 24-hr exposure liquid application

TXP Residue 24 hour wash					
Replicate Number	1	2	3	**Mean**	**StDev**
Applied (µg)	29.76	31.85	32.43	**31.35**	**1.40**
Recovered in swabs (µg)	12.99	15.64	15.89	**14.84**	**1.61**
Total recovery (%)	72.84	78.13	76.93	**75.97**	**2.77**
Donor wash (µg)	0.39	0.72	0.88	**0.66**	**0.25**
Disposition					
SC tape-strips 1-2 (µg)	0.25	0.17	0.25	**0.23**	**0.05**
SC tape-strips 3-15 (µg)	0.37	0.07	0.23	**0.22**	**0.15**
SC tape-strips 1-15 (µg)	0.62	0.24	0.49	**0.45**	**0.20**
Remaining skin (µg)	0.50	0.51	0.48	**0.50**	**0.02**
Receptor phase at 2 hr (µg)	1.41	1.75	1.73	**1.63**	**0.19**
Receptor phase at 3 hr (µg)	1.62	2.00	2.05	**1.89**	**0.24**
Receptor phase at 4 hr (µg)	1.90	2.25	2.29	**2.15**	**0.22**
Receptor phase at 5 hr (µg)	2.04	2.60	2.63	**2.42**	**0.33**
Receptor phase at 6 hr (µg)	2.27	2.98	3.03	**2.76**	**0.42**
Receptor phase at 7 hr (µg)	2.29	3.03	3.15	**2.83**	**0.47**
Receptor phase at 8 hr (µg)	2.49	3.23	3.24	**2.99**	**0.43**
Receptor phase at 24 hr (µg)	4.39	5.99	5.47	**5.28**	**0.81**
Total uptake/absorption (µg)[#]	5.26	6.57	6.18	**6.00**	**0.67**
% uptake/absorption of amount applied	17.69	20.62	19.05	**19.12**	**1.46**

Table A1 24 - Full Results from Paper 2, TXP 24-hr exposure residue application

TXP Liquid 30µl						
Replicate Number	1	2	3	4	**Mean**	**StDev**
Applied (µg)	30	30	30	30	**30**	
Recovered in swabs (µg)	17.24	19.34	20.05	15.47	**18.02**	**2.08**
Total recovery (%)	79.80	83.40	93.64	74.28	**82.78**	**8.15**
Donor wash (µg)	1.00	0.35	1.08	0.79	**0.81**	**0.33**
Disposition						
SC tape-strips 1-2 (µg)	0.87	0.77	1.76	0.81	**1.05**	**0.48**
SC tape-strips 3-15 (µg)	0.68	0.38	0.28	0.25	**0.40**	**0.20**
SC tape-strips 1-15 (µg)	1.55	1.14	2.04	1.05	**1.45**	**0.45**
Remaining skin (µg)	0.96	0.86	1.57	1.43	**1.20**	**0.35**
Receptor phase at 2 hr (µg)	1.59	1.40	1.20	1.07	**1.31**	**0.23**
Receptor phase at 3 hr (µg)	1.75	1.84	1.58	1.44	**1.65**	**0.18**
Receptor phase at 4 hr (µg)	2.12	2.25	1.73	1.76	**1.97**	**0.26**
Receptor phase at 5 hr (µg)	2.16	2.22	1.82	2.02	**2.06**	**0.18**
Receptor phase at 6 hr (µg)	2.09	2.39	2.05	2.21	**2.19**	**0.15**
Receptor phase at 7 hr (µg)	2.28	2.43	2.14	2.32	**2.29**	**0.12**
Receptor phase at 8 hr (µg)	2.29	2.51	2.48	2.42	**2.43**	**0.10**
Receptor phase at 24 hr (µg)	3.18	3.33	3.36	3.53	**3.35**	**0.14**
Total uptake/absorption (µg)[#]	4.82	4.57	5.10	5.12	**4.90**	**0.26**
% uptake/absorption of amount applied	16.08	15.23	17.00	17.08	**16.35**	**0.87**

Table A1 25 - Full Results from Paper 3, TXP 30 µl liquid loading dose

TXP Residue 40µg disc					
Replicate Number	1	2	3	**Mean**	**StDev**
Applied (µg)	25.27	24.60	25.21	**25.03**	**0.37**
Recovered in swabs (µg)	17.24	16.91	18.69	**17.61**	**0.95**
Total recovery (%)	85.98	85.18	89.71	**86.96**	**2.42**
Donor wash (µg)	0.13	< LOQ	0.13	**0.09**	**0.08**
Disposition					
SC tape-strips 1-2 (µg)	0.43	0.35	0.58	**0.45**	**0.12**
SC tape-strips 3-15 (µg)	0.18	0.16	0.21	**0.18**	**0.03**
SC tape-strips 1-15 (µg)	0.60	0.51	0.79	**0.63**	**0.14**
Remaining skin (µg)	0.57	0.47	0.50	**0.51**	**0.05**
Receptor phase at 2 hr (µg)	< LOQ	< LOQ	< LOQ	**< LOQ**	**< LOQ**
Receptor phase at 3 hr (µg)	< LOQ	< LOQ	< LOQ	**< LOQ**	**< LOQ**
Receptor phase at 4 hr (µg)	0.30	< LOQ	< LOQ	**0.10**	**0.17**
Receptor phase at 5 hr (µg)	0.39	< LOQ	< LOQ	**0.13**	**0.23**
Receptor phase at 6 hr (µg)	0.44	< LOQ	< LOQ	**0.15**	**0.25**
Receptor phase at 7 hr (µg)	0.50	< LOQ	< LOQ	**0.17**	**0.29**
Receptor phase at 8 hr (µg)	0.63	< LOQ	0.21	**0.28**	**0.32**
Receptor phase at 24 hr (µg)	1.13	0.79	0.98	**0.96**	**0.17**
Total uptake/absorption (µg)[#]	1.87	1.35	1.60	**1.61**	**0.26**
% uptake/absorption of amount applied	7.40	5.51	6.33	**6.41**	**0.95**

Table A1 26 - Full Results from Paper 3, TXP 40µg disc residue loading dose

TXP Liquid 70µl						
Replicate Number	1	2	3	4	**Mean**	**StDev**
Applied (µg)	70	70	70	70	**70**	
Recovered in swabs (µg)	46.88	34.85	48.08	47.36	**44.29**	**6.31**
Total recovery (%)	85.98	74.54	89.80	82.99	**83.33**	**6.48**
Donor wash (µg)	0.59	1.20	0.41	0.83	**0.76**	**0.34**
Disposition						
SC tape-strips 1-2 (µg)	1.11	1.74	1.41	1.00	**1.32**	**0.33**
SC tape-strips 3-15 (µg)	0.65	0.83	0.79	0.47	**0.69**	**0.16**
SC tape-strips 1-15 (µg)	1.77	2.57	2.20	1.47	**2.00**	**0.48**
Remaining skin (µg)	2.73	2.61	2.07	2.52	**2.49**	**0.29**
Receptor phase at 2 hr (µg)	3.63	4.98	3.22	2.18	**3.50**	**1.16**
Receptor phase at 3 hr (µg)	4.84	5.75	5.08	2.84	**4.63**	**1.26**
Receptor phase at 4 hr (µg)	6.01	7.98	6.37	3.17	**5.88**	**2.00**
Receptor phase at 5 hr (µg)	5.98	7.71	6.66	4.41	**6.19**	**1.38**
Receptor phase at 6 hr (µg)	6.45	8.23	7.12	3.67	**6.37**	**1.94**
Receptor phase at 7 hr (µg)	6.53	8.11	7.34	4.70	**6.67**	**1.46**
Receptor phase at 8 hr (µg)	7.75	8.78	8.06	5.21	**7.45**	**1.56**
Receptor phase at 24 hr (µg)	8.22	10.94	10.09	5.91	**8.79**	**2.23**
Total uptake/absorption (µg)[#]	11.60	14.39	12.96	8.91	**11.96**	**2.34**
% uptake/absorption of amount applied	16.57	20.56	18.51	12.72	**17.09**	**3.34**

Table A1 27 - Full Results from Paper 3, TXP 70 µl liquid loading dose

TXP Residue 100µg disc						
Replicate Number	1	2	3	4	**Mean**	**StDev**
Applied (µg)	68.79	63.51	69.89	65.47	**66.91**	**2.95**
Recovered in swabs (µg)	47.94	49.87	55.68	51.24	**51.18**	**3.29**
Total recovery (%)	86.21	93.97	90.44	91.04	**90.42**	**3.20**
Donor wash (µg)	0.37	2.16	0.24	0.40	**0.79**	**0.91**
Disposition						
SC tape-strips 1-2 (µg)	0.75	1.05	1.00	1.25	**1.01**	**0.21**
SC tape-strips 3-15 (µg)	0.20	0.39	0.44	0.59	**0.41**	**0.16**
SC tape-strips 1-15 (µg)	0.95	1.44	1.44	1.84	**1.42**	**0.37**
Remaining skin (µg)	1.21	1.59	0.83	1.52	**1.29**	**0.35**
Receptor phase at 2 hr (µg)	0.82	< LOQ	< LOQ	< LOQ	**0.20**	**0.41**
Receptor phase at 3 hr (µg)	1.11	0.27	0.25	< LOQ	**0.41**	**0.48**
Receptor phase at 4 hr (µg)	1.94	0.57	0.49	0.17	**0.79**	**0.79**
Receptor phase at 5 hr (µg)	2.03	0.65	0.60	0.23	**0.88**	**0.79**
Receptor phase at 6 hr (µg)	2.47	0.83	0.85	0.33	**1.12**	**0.93**
Receptor phase at 7 hr (µg)	2.72	0.96	0.93	0.36	**1.24**	**1.02**
Receptor phase at 8 hr (µg)	2.93	1.06	1.03	0.48	**1.38**	**1.07**
Receptor phase at 24 hr (µg)	4.53	2.42	2.14	1.51	**2.65**	**1.31**
Total uptake/absorption (µg)[#]	5.95	4.40	3.41	3.46	**4.30**	**1.19**
% uptake/absorption of amount applied	8.64	6.93	4.88	5.28	**6.44**	**1.72**

Table A1 28 - Full Results from Paper 3, TXP 100µg disc residue loading dose

TXP Liquid 120µl						
Replicate Number	1	2	3	4	**Mean**	**StDev**
Applied (µg)	120	120	120	120	**120**	
Recovered in swabs (µg)	59.32	72.81	80.00	74.54	**71.67**	**8.78**
Total recovery (%)	69.36	84.10	86.07	80.13	**79.92**	**7.46**
Donor wash (µg)	2.41	3.32	1.31	2.72	**2.44**	**0.84**
Disposition						
SC tape-strips 1-2 (µg)	0.75	1.94	1.83	1.75	**1.57**	**0.55**
SC tape-strips 3-15 (µg)	0.98	1.46	1.01	0.94	**1.10**	**0.25**
SC tape-strips 1-15 (µg)	1.72	3.40	2.84	2.69	**2.66**	**0.70**
Remaining skin (µg)	6.02	8.59	4.93	4.38	**5.98**	**1.87**
Receptor phase at 2 hr (µg)	5.47	5.66	7.00	3.93	**5.51**	**1.26**
Receptor phase at 3 hr (µg)	8.30	7.45	9.03	5.61	**7.60**	**1.47**
Receptor phase at 4 hr (µg)	10.76	9.03	10.27	6.49	**9.14**	**1.91**
Receptor phase at 5 hr (µg)	10.99	9.26	10.20	7.36	**9.45**	**1.56**
Receptor phase at 6 hr (µg)	11.41	9.20	11.00	8.52	**10.03**	**1.39**
Receptor phase at 7 hr (µg)	11.49	9.95	11.31	9.08	**10.46**	**1.15**
Receptor phase at 8 hr (µg)	11.40	9.62	11.77	9.40	**10.55**	**1.21**
Receptor phase at 24 hr (µg)	13.76	12.80	14.20	11.83	**13.15**	**1.06**
Total uptake/absorption (µg)[#]	20.75	22.86	20.14	17.15	**20.23**	**2.36**
% uptake/absorption of amount applied	17.29	19.05	16.79	14.29	**16.86**	**1.96**

Table A1 29 - Full Results from Paper 3, TXP 120 µl liquid loading dose

TXP Residue 180µg disc						
Replicate Number	1	2	3	4	**Mean**	**StDev**
Applied (µg)	123.65	125.86	121.70	108.31	**119.88**	**7.90**
Recovered in swabs (µg)	104.12	104.12	102.21	89.16	**99.90**	**7.22**
Total recovery (%)	94.00	95.49	94.14	93.99	**94.41**	**0.73**
Donor wash (µg)	0.51	1.12	0.37	0.89	**0.72**	**0.35**
Disposition						
SC tape-strips 1-2 (µg)	1.92	1.85	1.45	1.24	**1.62**	**0.32**
SC tape-strips 3-15 (µg)	0.76	0.90	0.60	0.38	**0.66**	**0.22**
SC tape-strips 1-15 (µg)	2.68	2.75	2.05	1.68	**2.29**	**0.51**
Remaining skin (µg)	0.82	2.75	1.54	3.28	**2.10**	**1.12**
Receptor phase at 2 hr (µg)	0.77	0.54	0.40	0.20	**0.48**	**0.24**
Receptor phase at 3 hr (µg)	1.12	0.85	0.63	0.27	**0.72**	**0.36**
Receptor phase at 4 hr (µg)	1.96	1.88	1.30	0.36	**1.37**	**0.74**
Receptor phase at 5 hr (µg)	2.23	2.53	1.63	0.48	**1.72**	**0.91**
Receptor phase at 6 hr (µg)	2.57	2.93	1.96	0.64	**2.03**	**1.01**
Receptor phase at 7 hr (µg)	2.80	3.31	2.23	0.88	**2.30**	**1.05**
Receptor phase at 8 hr (µg)	3.13	3.79	2.53	1.03	**2.62**	**1.18**
Receptor phase at 24 hr (µg)	5.33	7.46	5.58	3.07	**5.36**	**1.80**
Total uptake/absorption (µg)[#]	6.90	11.11	7.72	6.73	**8.11**	**2.04**
% uptake/absorption of amount applied	5.58	8.83	6.34	6.21	**6.74**	**1.43**

Table A1 30 - Full Results from Paper 3, TXP 180µg disc residue loading dose

CFL Liquid 30µl							
Replicate Number	1	2	3	4	5	**Mean**	**StDev**
Applied (µg)	30	30	30	30	30	**30**	
Recovered in swabs (µg)	22.38	21.81	22.17	20.05	24.98	**22.28**	**1.77**
Total recovery (%)	86.08	87.97	88.20	81.34	98.38	**88.39**	**6.23**
Donor wash (µg)	0.83	0.75	0.64	0.92	0.31	**0.69**	**0.24**
Disposition							
SC tape-strips 1-2 (µg)	1.94	2.56	1.71	1.89	3.05	**2.23**	**0.56**
SC tape-strips 3-15 (µg)	0.19	0.93	0.54	0.60	0.52	**0.56**	**0.26**
SC tape-strips 1-15 (µg)	2.14	3.49	2.24	2.49	3.53	**2.78**	**0.68**
Remaining skin (µg)	0.47	0.35	1.41	0.93	0.69	**0.77**	**0.42**
Receptor phase at 24 hr (µg)	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	**< LOQ**	**< LOQ**
Total uptake/absorption (µg)[#]	0.67	1.28	1.95	1.53	1.17	**1.32**	**0.47**
% uptake/absorption of amount applied	2.22	4.25	6.49	5.11	3.90	**4.40**	**1.57**

Table A1 31 - Full Results from Paper 3, CFL 30 µl liquid loading dose

CFL Residue 40µg disc					
Replicate Number	1	2	3	**Mean**	**StDev**
Applied (µg)	24.33	26.57	29.06	**26.65**	**2.36**
Recovered in swabs (µg)	24.29	23.20	19.91	**22.47**	**2.28**
Total recovery (%)	102.42	96.87	84.67	**94.65**	**9.08**
Donor wash (µg)	0.51	0.64	0.72	**0.62**	**0.10**
Disposition					
SC tape-strips 1-2 (µg)	0.49	1.32	2.07	**1.29**	**0.79**
SC tape-strips 3-15 (µg)	0.39	0.78	1.82	**1.00**	**0.74**
SC tape-strips 1-15 (µg)	0.10	0.54	0.24	**0.29**	**0.22**
Remaining skin (µg)	< LOQ	0.16	0.23	**0.13**	**0.12**
Receptor phase at 24 hr (µg)	< LOQ	< LOQ	< LOQ	**< LOQ**	**< LOQ**
Total uptake/absorption (µg)[#]	0.10	0.70	0.47	**0.42**	**0.30**
% uptake/absorption of amount applied	0.43	2.63	1.62	**1.56**	**1.11**

Table A1 32 - Full Results from Paper 3, CFL 40µg disc residue loading dose

CFL Liquid 70µl									
Replicate Number	1	2	3	4	5	6	7	**Mean**	**StDev**
Applied (µg)	70	70	70	70	70	70	70	**70**	
Recovered in swabs (µg)	63.72	56.46	54.52	56.12	45.98	51.28	52.72	**54.40**	**5.44**
Total recovery (%)	100.72	91.28	91.46	93.82	75.78	83.28	82.63	**88.42**	**8.34**
Donor wash (µg)	0.98	0.91	2.44	2.61	1.84	< LOQ	1.82	**1.51**	**0.93**
Disposition									
SC tape-strips 1-2 (µg)	4.26	4.38	4.89	4.65	2.02	4.61	2.20	**3.86**	**1.21**
SC tape-strips 3-15 (µg)	1.32	1.57	1.97	1.72	1.58	1.28	0.83	**1.46**	**0.36**
SC tape-strips 1-15 (µg)	5.59	5.94	6.85	6.37	3.60	5.89	3.03	**5.32**	**1.44**
Remaining skin (µg)	0.21	0.57	0.21	0.57	1.63	1.13	0.28	**0.66**	**0.54**
Receptor phase at 24 hr (µg)	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	**< LOQ**	**< LOQ**
Total uptake/absorption (µg)[#]	1.54	2.14	2.17	2.29	3.21	2.40	1.11	**2.12**	**0.67**
% uptake/absorption of amount applied	2.19	3.06	3.10	3.27	4.58	3.43	1.58	**3.03**	**0.95**

Table A1 33 - Full Results from Paper 3, CFL 70 µl liquid loading dose

CFL Residue 100µg disc									
Replicate Number	1	2	3	4	5	6	7	**Mean**	**StDev**
Applied (µg)	67.88	64.31	65.21	64.44	69.55	69.44	69.51	**67.19**	**2.46**
Recovered in swabs (µg)	56.31	50.46	48.67	48.93	54.19	56.87	58.07	**53.36**	**3.96**
Total recovery (%)	91.86	90.79	87.58	88.84	89.23	92.18	94.09	**90.65**	**2.25**
Donor wash (µg)	0.32	1.55	1.07	0.52	1.10	1.25	1.38	**1.03**	**0.45**
Disposition									
SC tape-strips 1-2 (µg)	1.39	1.17	2.15	1.97	1.53	2.00	2.29	**1.79**	**0.42**
SC tape-strips 3-15 (µg)	0.48	0.15	0.31	0.33	0.77	0.67	0.44	**0.45**	**0.22**
SC tape-strips 1-15 (µg)	1.88	1.32	2.47	2.29	2.30	2.67	2.72	**2.24**	**0.49**
Remaining skin (µg)	1.23	1.76	0.58	1.53	1.19	0.83	1.42	**1.22**	**0.41**
Receptor phase at 24 hr (µg)	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	**< LOQ**	**< LOQ**
Total uptake/absorption (µg)[#]	1.72	1.91	0.89	1.86	1.96	1.50	1.86	**1.67**	**0.38**
% uptake/absorption of amount applied	2.53	2.98	1.37	2.88	2.82	2.16	2.68	**2.49**	**0.56**

Table A1 34 - Full Results from Paper 3, CFL 100µg disc residue loading dose

CFL Liquid 120µl								
Replicate Number	1	2	3	4	5	6	**Mean**	**StDev**
Applied (µg)	120	120	120	120	120	120	**120**	
Recovered in swabs (µg)	99.29	103.72	88.28	93.23	99.02	100.44	**97.33**	**5.58**
Total recovery (%)	89.66	94.71	80.51	85.98	91.85	95.86	**89.76**	**5.76**
Donor wash (µg)	< LOQ	1.95	2.45	2.90	2.03	6.54	**2.64**	**2.15**
Disposition								
SC tape-strips 1-2 (µg)	6.06	5.68	2.32	1.88	5.44	3.47	**4.14**	**1.83**
SC tape-strips 3-15 (µg)	0.67	1.77	1.39	1.79	1.40	0.73	**1.29**	**0.49**
SC tape-strips 1-15 (µg)	6.73	7.45	3.71	3.67	6.85	4.19	**5.43**	**1.75**
Remaining skin (µg)	1.58	0.54	2.18	3.37	2.32	3.85	**2.31**	**1.20**
Receptor phase at 24 hr (µg)	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	**< LOQ**	**< LOQ**
Total uptake/absorption (µg)[#]	2.24	2.31	3.57	5.17	3.73	4.58	**3.60**	**1.18**
% uptake/absorption of amount applied	1.87	1.93	2.98	4.30	3.11	3.82	**3.00**	**0.98**

Table A1 35 - Full Results from Paper 3, CFL 120 µl liquid loading dose

CFL Residue 180µg disc								
Replicate Number	1	2	3	4	5	6	**Mean**	**StDev**
Applied (µg)	137.69	141.69	129.26	129.48	121.64	123.23	**130.50**	**7.89**
Recovered in swabs (µg)	105.51	120.76	84.94	99.39	102.88	104.51	**103.00**	**11.52**
Total recovery (%)	87.52	92.66	81.93	87.64	94.35	93.12	**89.54**	**4.72**
Donor wash (µg)	1.63	2.29	4.05	3.19	4.36	1.32	**2.81**	**1.26**
Disposition								
SC tape-strips 1-2 (µg)	3.94	3.63	4.17	2.16	1.87	3.57	**3.22**	**0.97**
SC tape-strips 3-15 (µg)	1.49	0.96	1.80	1.21	0.89	1.64	**1.33**	**0.37**
SC tape-strips 1-15 (µg)	5.43	4.59	5.97	3.38	2.75	4.89	**4.50**	**1.23**
Remaining skin (µg)	2.65	0.84	1.78	1.27	1.47	0.32	**1.39**	**0.80**
Receptor phase at 24 hr (µg)	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	**< LOQ**	**< LOQ**
Total uptake/absorption (µg)[#]	4.14	1.80	3.58	2.49	2.35	1.64	**2.67**	**0.99**
% uptake/absorption of amount applied	3.01	1.27	2.77	1.92	1.94	1.33	**2.04**	**0.72**

Table A1 36 - Full Results from Paper 3, CFL 180 µg disc residue loading dose

CFL Liquid 160µl					
Replicate Number	1	2	3	**Mean**	**StDev**
Applied (µg)	160	160	160	**160**	
Recovered in swabs (µg)	136.67	139.58	142.99	**139.75**	**3.16**
Total recovery (%)	91.21	93.71	94.17	**93.03**	**1.60**
Donor wash (µg)	2.22	1.92	< LOQ	**1.38**	**1.20**
Disposition					
SC tape-strips 1-2 (µg)	3.32	4.45	4.36	**4.04**	**0.62**
SC tape-strips 3-15 (µg)	0.64	1.24	0.95	**0.94**	**0.30**
SC tape-strips 1-15 (µg)	3.96	5.69	5.31	**4.99**	**0.91**
Remaining skin (µg)	3.08	2.74	2.38	**2.73**	**0.35**
Receptor phase at 24 hr (µg)	< LOQ	< LOQ	< LOQ	**< LOQ**	**< LOQ**
Total uptake/absorption (µg)[#]	3.72	3.98	3.33	**3.68**	**0.33**
% uptake/absorption of amount applied	2.33	2.49	2.08	**2.30**	**0.21**

Table A1 37 - Full Results from Paper 3, CFL 160 µl liquid loading dose

CFL Residue 250µg disc					
Replicate Number	1	2	3	**Mean**	**StDev**
Applied (µg)	165.93	171.48	170.81	**169.41**	**3.03**
Recovered in swabs (µg)	154.93	154.75	150.63	**153.43**	**2.43**
Total recovery (%)	94.88	93.10	92.02	**93.33**	**1.44**
Donor wash (µg)	2.98	2.89	2.14	**2.67**	**0.46**
Disposition					
SC tape-strips 1-2 (µg)	2.18	2.36	3.75	**2.76**	**0.86**
SC tape-strips 3-15 (µg)	1.32	0.95	0.79	**1.02**	**0.27**
SC tape-strips 1-15 (µg)	3.50	3.31	4.54	**3.78**	**0.67**
Remaining skin (µg)	1.20	2.61	2.76	**2.19**	**0.86**
Receptor phase at 24 hr (µg)	< LOQ	< LOQ	< LOQ	**< LOQ**	**< LOQ**
Total uptake/absorption (µg)[#]	2.52	3.56	3.55	**3.21**	**0.60**
% uptake/absorption of amount applied	1.52	2.07	2.08	**1.89**	**0.32**

Table A1 38 - Full Results from Paper 3, CFL 250 µg disc residue loading dose

TXP Liquid EC- A							
Replicate Number	1	2	3	4	5	**Mean**	**StDev**
Applied (µg)	30	30	30	30	30	**30**	
Recovered in swabs (µg)	19.83	19.29	20.28	19.02	15.79	**18.84**	**1.77**
Total recovery (%)	84.80	88.00	89.42	93.54	79.09	**86.97**	**5.41**
Donor wash (µg)	0.25	0.48	0.19	0.92	0.46	**0.46**	**0.29**
Disposition							
SC tape-strips 1-2 (µg)	0.93	1.08	1.00	1.67	0.82	**1.10**	**0.33**
SC tape-strips 3-15 (µg)	0.39	0.66	0.35	1.15	0.19	**0.55**	**0.38**
SC tape-strips 1-15 (µg)	1.33	1.74	1.35	2.82	1.01	**1.65**	**0.70**
Remaining skin (µg)	1.25	1.46	1.16	0.81	0.80	**1.09**	**0.29**
Receptor phase at 2 hr (µg)	0.96	0.62	1.10	1.07	1.59	**1.07**	**0.35**
Receptor phase at 3 hr (µg)	1.40	1.10	1.70	1.91	2.48	**1.72**	**0.52**
Receptor phase at 4 hr (µg)	1.68	1.41	2.01	2.44	3.14	**2.14**	**0.68**
Receptor phase at 5 hr (µg)	1.82	1.66	2.28	3.06	3.76	**2.52**	**0.88**
Receptor phase at 6 hr (µg)	1.99	1.97	2.55	3.37	4.03	**2.78**	**0.90**
Receptor phase at 7 hr (µg)	2.09	2.19	2.67	3.45	4.28	**2.93**	**0.92**
Receptor phase at 8 hr (µg)	2.24	2.29	2.71	3.58	4.64	**3.09**	**1.02**
Receptor phase at 24 hr (µg)	2.79	3.43	3.85	4.49	5.66	**4.04**	**1.09**
Total uptake/absorption (µg)[#]	4.43	5.55	5.35	6.45	6.65	**5.69**	**0.90**
% uptake/absorption of amount applied	14.76	18.50	17.85	21.50	22.17	**18.95**	**2.99**

Table A1 39 - Full Results from Paper 3, TXP 30µg liquid EC – A formulation

TXP Residue EC- A							
Replicate Number	1	2	3	4	5	**Mean**	**StDev**
Applied (µg)	25.19	28.68	25.59	27.99	27.89	**27.07**	**1.57**
Recovered in swabs (µg)	24.35	19.42	16.87	18.86	14.88	**18.87**	**3.54**
Total recovery (%)	109.56	92.23	84.12	84.45	79.66	**90.01**	**11.83**
Donor wash (µg)	0.18	0.34	0.13	0.18	0.24	**0.21**	**0.08**
Disposition							
SC tape-strips 1-2 (µg)	1.02	0.64	0.42	0.48	0.75	**0.66**	**0.24**
SC tape-strips 3-15 (µg)	0.47	0.32	0.17	0.13	0.35	**0.29**	**0.14**
SC tape-strips 1-15 (µg)	1.49	0.96	0.59	0.61	1.10	**0.95**	**0.38**
Remaining skin (µg)	0.92	0.32	0.55	0.66	1.36	**0.76**	**0.40**
Receptor phase at 2 hr (µg)	0.67	2.17	< LOQ	< LOQ	0.77	**0.72**	**0.89**
Receptor phase at 3 hr (µg)	0.90	2.71	< LOQ	0.27	1.05	**0.99**	**1.06**
Receptor phase at 4 hr (µg)	1.11	2.93	0.29	0.59	1.23	**1.23**	**1.02**
Receptor phase at 5 hr (µg)	1.22	3.17	0.38	0.65	1.31	**1.35**	**1.09**
Receptor phase at 6 hr (µg)	1.34	3.62	0.43	0.68	1.40	**1.49**	**1.26**
Receptor phase at 7 hr (µg)	1.34	3.66	0.49	0.84	1.48	**1.56**	**1.24**
Receptor phase at 8 hr (µg)	1.43	3.79	0.61	0.85	1.52	**1.64**	**1.26**
Receptor phase at 24 hr (µg)	2.07	4.53	1.10	1.47	2.18	**2.27**	**1.34**
Total uptake/absorption (µg)[#]	3.46	5.17	1.83	2.26	3.88	**3.32**	**1.33**
% uptake/absorption of amount applied	13.75	18.03	7.14	8.07	13.93	**12.18**	**4.53**

Table A1 40 - Full Results from Paper 3, TXP 40µg disc residue EC- A formulation

TXP Liquid EC- B						
Replicate Number	1	2	3	4	**Mean**	**StDev**
Applied (µg)	30	30	30	30	**30**	
Recovered in swabs (µg)	16.84	15.71	15.84	19.89	**17.07**	**1.95**
Total recovery (%)	82.91	76.79	76.83	84.67	**80.30**	**4.10**
Donor wash (µg)	1.11	0.92	0.90	0.35	**0.82**	**0.33**
Disposition						
SC tape-strips 1-2 (µg)	0.75	0.97	0.70	0.87	**0.82**	**0.12**
SC tape-strips 3-15 (µg)	0.83	0.69	0.39	0.34	**0.56**	**0.24**
SC tape-strips 1-15 (µg)	1.58	1.66	1.09	1.21	**1.39**	**0.28**
Remaining skin (µg)	1.53	1.06	1.49	0.65	**1.18**	**0.41**
Receptor phase at 2 hr (µg)	0.84	1.05	0.74	< LOQ	**0.66**	**0.46**
Receptor phase at 3 hr (µg)	1.46	1.45	1.31	1.30	**1.38**	**0.09**
Receptor phase at 4 hr (µg)	1.92	1.75	1.79	1.48	**1.73**	**0.18**
Receptor phase at 5 hr (µg)	2.16	1.89	1.95	1.65	**1.91**	**0.21**
Receptor phase at 6 hr (µg)	2.46	2.20	2.26	1.90	**2.20**	**0.23**
Receptor phase at 7 hr (µg)	1.97	2.36	2.52	2.13	**2.24**	**0.25**
Receptor phase at 8 hr (µg)	2.72	2.47	2.68	2.16	**2.51**	**0.26**
Receptor phase at 24 hr (µg)	3.81	3.68	3.73	3.29	**3.63**	**0.23**
Total uptake/absorption (µg)[#]	6.17	5.43	5.61	4.29	**5.38**	**0.79**
% uptake/absorption of amount applied	20.58	18.10	18.71	14.30	**17.92**	**2.63**

Table A1 41 - A41 Full Results from Paper 3, TXP 30µg liquid EC – B formulation

TXP Residue EC- B									
Replicate Number	1	2	3	4	5	6	7	**Mean**	**StDev**
Applied (µg)	26.41	30.17	25.19	26.24	31.49	31.48	32.86	**29.12**	**3.09**
Recovered in swabs (µg)	15.28	14.94	11.71	11.14	16.20	14.16	16.52	**14.28**	**2.11**
Total recovery (%)	81.69	75.72	76.20	76.57	75.45	71.03	75.13	**75.97**	**3.12**
Donor wash (µg)	0.49	0.21	0.33	0.27	0.78	0.71	0.44	**0.46**	**0.22**
Disposition									
SC tape-strips 1-2 (µg)	0.63	0.75	0.80	0.98	0.70	1.51	0.54	**0.85**	**0.32**
SC tape-strips 3-15 (µg)	0.33	0.56	0.45	0.37	0.49	0.54	0.15	**0.41**	**0.14**
SC tape-strips 1-15 (µg)	0.95	1.31	1.25	1.35	1.19	2.05	0.70	**1.26**	**0.42**
Remaining skin (µg)	0.48	0.39	0.30	0.89	0.55	0.62	2.04	**0.75**	**0.60**
Receptor phase at 2 hr (µg)	< LOQ	1.32	0.56	0.62	0.95	0.70	0.70	**0.69**	**0.40**
Receptor phase at 3 hr (µg)	0.67	1.85	0.83	1.12	1.11	0.82	1.03	**1.06**	**0.39**
Receptor phase at 4 hr (µg)	0.87	2.11	0.97	1.40	1.31	0.95	1.20	**1.26**	**0.43**
Receptor phase at 5 hr (µg)	1.01	2.36	1.25	1.70	1.45	1.09	1.38	**1.46**	**0.46**
Receptor phase at 6 hr (µg)	1.11	2.76	1.37	2.05	1.69	1.28	1.52	**1.68**	**0.56**
Receptor phase at 7 hr (µg)	1.27	2.84	1.48	2.23	1.79	1.38	1.79	**1.83**	**0.55**
Receptor phase at 8 hr (µg)	1.52	3.13	1.69	2.35	1.99	1.54	1.78	**2.00**	**0.58**
Receptor phase at 24 hr (µg)	1.89	3.61	2.07	3.21	2.95	2.35	3.22	**2.76**	**0.66**
Total uptake/absorption (µg)[#]	2.69	4.56	2.82	4.48	3.99	3.51	5.41	**3.92**	**0.99**
% uptake/absorption of amount applied	10.19	15.10	11.18	17.07	12.66	11.15	16.46	**13.40**	**2.79**

Table A1 42 - Full Results from Paper 3, TXP 40µg disc residue EC- B formulation

TXP Liquid WP							
Replicate Number	1	2	3	4	5	**Mean**	**StDev**
Applied (µg)	30	30	30	30	30	**30**	
Recovered in swabs (µg)	21.05	20.60	19.96	20.72	19.02	**20.27**	**0.80**
Total recovery (%)	86.18	85.14	85.12	84.95	76.18	**83.52**	**4.13**
Donor wash (µg)	0.28	0.69	0.60	0.39	1.11	**0.61**	**0.32**
Disposition							
SC tape-strips 1-2 (µg)	1.10	0.82	1.01	1.08	0.84	**0.97**	**0.13**
SC tape-strips 3-15 (µg)	0.67	0.44	0.53	0.35	0.15	**0.43**	**0.20**
SC tape-strips 1-15 (µg)	1.77	1.26	1.55	1.43	0.99	**1.40**	**0.29**
Remaining skin (µg)	0.86	1.03	0.96	1.06	0.46	**0.87**	**0.25**
Receptor phase at 2 hr (µg)	0.47	0.47	0.67	0.58	0.33	**0.50**	**0.13**
Receptor phase at 3 hr (µg)	0.81	0.70	0.92	0.86	0.53	**0.76**	**0.15**
Receptor phase at 4 hr (µg)	1.01	0.90	1.08	1.22	0.69	**0.98**	**0.20**
Receptor phase at 5 hr (µg)	1.20	1.03	1.48	1.27	0.81	**1.16**	**0.25**
Receptor phase at 6 hr (µg)	1.27	1.12	1.59	1.43	0.95	**1.27**	**0.25**
Receptor phase at 7 hr (µg)	1.29	1.15	1.45	1.47	1.00	**1.27**	**0.20**
Receptor phase at 8 hr (µg)	1.30	1.27	1.64	1.54	1.00	**1.35**	**0.25**
Receptor phase at 24 hr (µg)	1.90	1.95	2.46	1.89	1.28	**1.90**	**0.42**
Total uptake/absorption (µg)[#]	3.43	3.42	3.96	3.29	1.88	**3.20**	**0.78**
% uptake/absorption of amount applied	11.42	11.41	13.20	10.97	6.27	**10.65**	**2.59**

Table A1 43 - Full Results from Paper 3, TXP 30 µg liquid WP formulation

TXP Residue WP											
Replicate Number	1	2	3	4	5	6	7	8	9	**Mean**	**StDev**
Applied (µg)	22.25	14.29	27.87	31.64	26.92	29.17	30.89	31.26	31.26	**27.28**	**5.72**
Recovered in swabs (µg)	15.11	8.31	13.15	18.37	14.77	17.93	18.29	18.98	16.72	**15.74**	**3.41**
Total recovery (%)	89.73	88.71	82.19	80.69	83.08	81.93	78.12	81.38	76.31	**82.46**	**4.38**
Donor wash (µg)	0.34	0.30	0.43	0.40	0.63	0.76	0.56	0.43	0.88	**0.52**	**0.20**
Disposition											
SC tape-strips 1-2 (µg)	0.61	0.35	3.21	1.40	1.11	0.63	0.85	0.94	1.36	**1.16**	**0.84**
SC tape-strips 3-15 (µg)	0.24	0.12	0.42	0.83	0.24	0.28	0.44	0.44	0.35	**0.37**	**0.20**
SC tape-strips 1-15 (µg)	0.85	0.47	3.62	2.22	1.36	0.91	1.29	1.37	1.72	**1.54**	**0.93**
Remaining skin (µg)	0.61	0.35	0.12	0.75	1.13	0.80	0.55	0.46	0.72	**0.61**	**0.29**
Receptor phase at 2 hr (µg)	0.41	< LOQ	1.64	0.46	0.71	0.66	0.62	1.05	0.84	**0.71**	**0.46**
Receptor phase at 3 hr (µg)	0.56	< LOQ	2.02	0.79	1.03	0.91	0.83	1.34	1.07	**0.95**	**0.55**
Receptor phase at 4 hr (µg)	0.71	< LOQ	2.25	0.97	1.21	0.99	0.90	1.53	1.09	**1.07**	**0.61**
Receptor phase at 5 hr (µg)	0.82	0.20	2.43	1.15	1.40	1.02	0.94	1.69	1.15	**1.20**	**0.62**
Receptor phase at 6 hr (µg)	0.86	0.20	2.69	1.37	1.62	1.09	1.02	1.69	1.23	**1.31**	**0.68**
Receptor phase at 7 hr (µg)	1.14	0.26	2.80	1.41	1.63	1.15	1.03	1.65	1.21	**1.36**	**0.68**
Receptor phase at 8 hr (µg)	0.96	0.27	2.84	1.53	1.79	1.19	1.02	1.85	1.35	**1.42**	**0.72**
Receptor phase at 24 hr (µg)	1.24	0.35	3.43	2.16	2.27	1.54	1.45	2.56	1.75	**1.86**	**0.88**
Total uptake/absorption (µg)[#]	2.08	0.82	3.96	3.74	3.64	2.62	2.44	3.46	2.82	**2.84**	**1.00**
% uptake/absorption of amount applied	9.36	5.71	14.21	11.83	13.53	8.97	7.90	11.07	10.12	**10.30**	**2.70**

Table A1 44 - A44 Full Results from Paper 3, TXP 40µg disc residue WP formulation

TXP Liquid AI						
Replicate Number	1	2	3	4	**Mean**	**StDev**
Applied (µg)	30	30	30	30	**30**	
Recovered in swabs (µg)	12.81	12.89	14.26	12.58	**13.13**	**0.76**
Total recovery (%)	64.07	71.24	65.61	58.72	**64.91**	**5.15**
Donor wash (µg)	0.88	0.72	0.63	0.71	**0.73**	**0.10**
Disposition						
SC tape-strips 1-2 (µg)	0.85	1.78	0.73	1.10	**1.12**	**0.47**
SC tape-strips 3-15 (µg)	0.36	0.42	0.26	0.41	**0.36**	**0.07**
SC tape-strips 1-15 (µg)	1.21	2.20	1.00	1.51	**1.48**	**0.53**
Remaining skin (µg)	0.91	1.02	1.75	1.06	**1.18**	**0.38**
Receptor phase at 2 hr (µg)	0.90	1.30	0.55	0.43	**0.80**	**0.39**
Receptor phase at 3 hr (µg)	1.42	2.08	0.86	0.65	**1.25**	**0.64**
Receptor phase at 4 hr (µg)	1.82	2.60	1.04	0.82	**1.57**	**0.81**
Receptor phase at 5 hr (µg)	2.06	2.91	1.16	0.87	**1.75**	**0.93**
Receptor phase at 6 hr (µg)	2.31	3.32	1.33	1.04	**2.00**	**1.04**
Receptor phase at 7 hr (µg)	2.47	3.50	1.41	1.17	**2.14**	**1.07**
Receptor phase at 8 hr (µg)	2.61	3.68	1.51	1.24	**2.26**	**1.12**
Receptor phase at 24 hr (µg)	3.42	4.54	2.05	1.76	**2.94**	**1.29**
Total uptake/absorption (µg)[#]	4.69	5.97	4.07	3.23	**4.49**	**1.16**
% uptake/absorption of amount applied	15.62	19.91	13.55	10.76	**14.96**	**3.85**

Table A1 45 - Full Results from Paper 3, TXP 30µg liquid AI formulation

TXP Residue AI					
Replicate Number	1	2	3	**Mean**	**StDev**
Applied (µg)	12.42	10.58	11.01	**11.34**	**0.96**
Recovered in swabs (µg)	3.02	2.59	4.41	**3.34**	**0.95**
Total recovery (%)	79.46	82.39	86.44	**82.76**	**3.50**
Donor wash (µg)	0.52	0.79	0.35	**0.55**	**0.22**
Disposition					
SC tape-strips 1-2 (µg)	0.19	0.10	0.30	**0.19**	**0.10**
SC tape-strips 3-15 (µg)	0.03	0.05	0.03	**0.04**	**0.01**
SC tape-strips 1-15 (µg)	0.22	0.15	0.33	**0.23**	**0.09**
Remaining skin (µg)	0.19	< LOQ	< LOQ	**0.06**	**0.11**
Receptor phase at 2 hr (µg)	< LOQ	< LOQ	< LOQ	**< LOQ**	**< LOQ**
Receptor phase at 3 hr (µg)	< LOQ	< LOQ	< LOQ	**< LOQ**	**< LOQ**
Receptor phase at 4 hr (µg)	< LOQ	< LOQ	< LOQ	**< LOQ**	**< LOQ**
Receptor phase at 5 hr (µg)	< LOQ	< LOQ	< LOQ	**< LOQ**	**< LOQ**
Receptor phase at 6 hr (µg)	< LOQ	< LOQ	< LOQ	**< LOQ**	**< LOQ**
Receptor phase at 7 hr (µg)	< LOQ	< LOQ	0.18	**0.06**	**0.11**
Receptor phase at 8 hr (µg)	< LOQ	< LOQ	0.27	**0.09**	**0.15**
Receptor phase at 24 hr (µg)	0.25	< LOQ	0.49	**0.25**	**0.25**
Total uptake/absorption (µg)[#]	0.47	0.05	0.53	**0.35**	**0.26**
% uptake/absorption of amount applied	3.82	0.48	4.80	**3.04**	**2.26**

Table A1 46 - Full Results from Paper 3, TXP 40µg disc residue AI formulation

CFL Liquid EC- A								
Replicate Number	1	2	3	4	5	6	**Mean**	**StDev**
Applied (µg)	30	30	30	30	30	30	**30**	
Recovered in swabs (µg)	21.67	21.49	17.12	21.78	22.08	26.06	**21.70**	**2.84**
Total recovery (%)	87.77	84.53	63.24	85.33	88.04	95.67	**84.10**	**10.95**
Donor wash (µg)	2.61	0.96	0.40	0.82	0.86	0.19	**0.97**	**0.85**
Disposition								
SC tape-strips 1-2 (µg)	1.30	1.54	0.54	1.14	1.99	1.00	**1.25**	**0.49**
SC tape-strips 3-15 (µg)	0.52	0.37	0.41	0.85	0.92	0.19	**0.54**	**0.29**
SC tape-strips 1-15 (µg)	1.82	1.91	0.96	1.99	2.91	1.19	**1.80**	**0.69**
Remaining skin (µg)	0.23	1.00	0.50	1.01	0.56	0.71	**0.67**	**0.30**
Receptor phase at 24 hr (µg)	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	**< LOQ**	**< LOQ**
Total uptake/absorption (µg)*	0.76	1.37	0.91	1.86	1.48	0.90	**1.21**	**0.43**
% uptake/absorption of amount applied	2.52	4.57	3.04	6.21	4.92	3.00	**4.04**	**1.42**

Table A1 47 - Full Results from Paper 3, CFL 30µg liquid EC – A formulation

CFL Residue EC- A								
Replicate Number	1	2	3	4	5	6	**Mean**	**StDev**
Applied (µg)	23.62	19.26	23.71	21.78	23.98	23.14	**22.58**	**1.81**
Recovered in swabs (µg)	20.32	13.73	19.40	19.90	17.01	18.57	**18.16**	**2.46**
Total recovery (%)	94.81	88.36	91.84	98.97	85.38	91.90	**91.88**	**4.77**
Donor wash (µg)	0.22	< LOQ	< LOQ	0.58	0.18	0.25	**0.20**	**0.21**
Disposition								
SC tape-strips 1-2 (µg)	0.43	0.53	0.45	0.38	0.50	0.57	**0.48**	**0.07**
SC tape-strips 3-15 (µg)	0.17	0.34	0.24	0.51	0.44	0.51	**0.37**	**0.14**
SC tape-strips 1-15 (µg)	0.60	0.87	0.69	0.88	0.94	1.08	**0.85**	**0.17**
Remaining skin (µg)	0.41	< LOQ	0.35	< LOQ	< LOQ	< LOQ	**0.13**	**0.20**
Receptor phase at 24 hr (µg)	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	**< LOQ**	**< LOQ**
Total uptake/absorption (µg)*	0.57	0.34	0.59	0.51	0.44	0.51	**0.49**	**0.09**
% uptake/absorption of amount applied	2.43	1.74	2.48	2.33	1.84	2.21	**2.17**	**0.31**

Table A1 48 - Full Results from Paper 3, CFL 40µg disc residue EC- A formulation

CFL Liquid EC- B								
Replicate Number	1	2	3	4	5	6	**Mean**	**StDev**
Applied (µg)	30	30	30	30	30	30	**30**	
Recovered in swabs (µg)	21.26	19.43	20.10	22.58	23.13	24.38	**21.82**	**1.89**
Total recovery (%)	84.71	75.06	83.37	88.12	89.11	89.93	**85.05**	**5.52**
Donor wash (µg)	1.24	1.09	2.33	0.92	0.50	0.35	**1.07**	**0.70**
Disposition								
SC tape-strips 1-2 (µg)	1.80	0.63	1.09	1.50	1.32	1.34	**1.28**	**0.40**
SC tape-strips 3-15 (µg)	0.84	0.61	0.87	0.29	0.67	0.34	**0.60**	**0.24**
SC tape-strips 1-15 (µg)	2.64	1.24	1.95	1.80	1.99	1.68	**1.88**	**0.46**
Remaining skin (µg)	0.27	0.76	0.63	1.14	1.10	0.58	**0.75**	**0.33**
Receptor phase at 24 hr (µg)	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	**< LOQ**	**< LOQ**
Total uptake/absorption (µg)[#]	1.11	1.37	1.50	1.44	1.78	0.91	**1.35**	**0.30**
% uptake/absorption of amount applied	3.71	4.57	4.99	4.79	5.92	3.05	**4.50**	**1.01**

Table A1 49 - Full Results from Paper 3, CFL 30µg liquid EC – B formulation

CFL Residue EC- B						
Replicate Number	1	2	3	4	**Mean**	**StDev**
Applied (µg)	24.43	25.84	26.93	27.43	**26.16**	**1.33**
Recovered in swabs (µg)	18.51	21.48	20.12	18.62	**19.68**	**1.41**
Total recovery (%)	89.49	92.23	86.93	81.75	**87.60**	**4.46**
Donor wash (µg)	0.40	0.30	0.50	0.37	**0.39**	**0.08**
Disposition						
SC tape-strips 1-2 (µg)	0.77	0.42	0.46	0.68	**0.58**	**0.17**
SC tape-strips 3-15 (µg)	0.40	0.30	0.50	0.19	**0.35**	**0.13**
SC tape-strips 1-15 (µg)	1.16	0.72	0.96	0.86	**0.93**	**0.19**
Remaining skin (µg)	0.16	0.23	0.13	0.28	**0.20**	**0.07**
Receptor phase at 24 hr (µg)	< LOQ	< LOQ	< LOQ	< LOQ	**< LOQ**	**< LOQ**
Total uptake/absorption (µg)[#]	0.56	0.53	0.63	0.46	**0.55**	**0.07**
% uptake/absorption of amount applied	2.27	2.06	2.33	1.69	**2.09**	**0.29**

Table A1 50 - Full Results from Paper 3, CFL 40µg disc residue EC- B formulation

CFL Liquid WP										
Replicate Number	1	2	3	4	5	6	7	8	**Mean**	**StDev**
Applied (µg)	30	30	30	30	30	30	30	30	**30**	
Recovered in swabs (µg)	23.72	23.73	24.70	23.70	26.18	23.30	29.77	22.28	**24.67**	**2.35**
Total recovery (%)	91.66	87.97	91.49	89.55	99.90	91.54	110.61	89.14	**93.98**	**7.64**
Donor wash (µg)	0.75	0.59	0.55	0.62	0.30	0.55	1.33	0.59	**0.66**	**0.30**
Disposition										
SC tape-strips 1-2 (µg)	1.93	1.09	1.41	1.72	2.48	2.41	1.19	2.02	**1.78**	**0.53**
SC tape-strips 3-15 (µg)	0.84	0.73	0.64	0.34	0.43	1.06	0.55	0.77	**0.67**	**0.23**
SC tape-strips 1-15 (µg)	2.77	1.82	2.06	2.06	2.91	3.47	1.73	2.80	**2.45**	**0.62**
Remaining skin (µg)	0.26	0.25	0.13	0.49	0.58	0.14	0.35	1.07	**0.41**	**0.31**
Receptor phase at 24 hr (µg)	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	**< LOQ**	**< LOQ**
Total uptake/absorption (µg)[#]	1.10	0.98	0.78	0.83	1.00	1.20	0.89	1.85	**1.08**	**0.34**
% uptake/absorption of amount applied	3.68	3.27	2.59	2.77	3.35	4.01	2.98	6.16	**3.60**	**1.13**

Table A1 51 - Full Results from Paper 3, CFL 30µg liquid WP formulation

CFL Residue WP										
Replicate Number	1	2	3	4	5	6	7	8	**Mean**	**StDev**
Applied (µg)	36.00	33.11	34.56	32.90	38.09	37.35	33.64	36.16	**35.23**	**1.96**
Recovered in swabs (µg)	24.19	24.98	37.64	29.31	39.37	35.58	31.48	33.45	**32.00**	**5.59**
Total recovery (%)	76.35	85.80	117.12	93.65	109.68	100.74	100.05	97.77	**97.65**	**12.81**
Donor wash (µg)	0.33	0.56	0.92	0.39	0.95	0.66	0.95	0.53	**0.66**	**0.25**
Disposition										
SC tape-strips 1-2 (µg)	1.27	1.36	2.12	0.27	1.34	0.88	0.66	0.75	**1.08**	**0.57**
SC tape-strips 3-15 (µg)	0.63	0.54	0.73	0.23	0.16	0.31	0.38	0.36	**0.42**	**0.20**
SC tape-strips 1-15 (µg)	1.90	1.89	2.85	0.50	1.50	1.19	1.04	1.11	**1.50**	**0.72**
Remaining skin (µg)	0.12	< LOQ	< LOQ	0.16	0.13	0.22	0.19	0.18	**0.12**	**0.08**
Receptor phase at 24 hr (µg)	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	**< LOQ**	**< LOQ**
Total uptake/absorption (µg)[#]	0.74	0.54	0.73	0.39	0.29	0.53	0.56	0.54	**0.54**	**0.15**
% uptake/absorption of amount applied	2.07	1.62	2.10	1.19	0.76	1.43	1.68	1.50	**1.54**	**0.44**

Table A1 52 - Full Results from Paper 3, CFL 40µg disc residue WP formulation

CFL Residue AI							
Replicate Number	1	2	3	4	5	**Mean**	**StDdev**
Applied (µg)	14.29	18.64	14.90	22.11	19.42	**17.87**	**3.26**
Recovered in swabs (µg)	7.04	8.93	7.78	13.29	11.74	**9.76**	**2.66**
Total recovery (%)	90.58	82.00	86.98	84.09	86.05	**85.94**	**3.22**
Donor wash (µg)	2.59	1.75	0.90	1.54	1.11	**1.58**	**0.66**
Disposition							
SC tape-strips 1-2 (µg)	0.53	0.24	0.51	0.48	0.58	**0.47**	**0.13**
SC tape-strips 3-15 (µg)	0.23	0.29	0.24	0.25	0.20	**0.24**	**0.03**
SC tape-strips 1-15 (µg)	0.77	0.53	0.76	0.73	0.79	**0.71**	**0.11**
Remaining skin (µg)	0.12	0.24	0.25	0.19	0.21	**0.20**	**0.05**
Receptor phase at 24 hr (µg)	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	**< LOQ**	**< LOQ**
Total uptake/absorption (µg)[#]	0.36	0.53	0.50	0.44	0.42	**0.45**	**0.07**
% uptake/absorption of amount applied	2.49	2.82	3.33	1.98	2.15	**2.55**	**0.54**

Table A1 53 - Full Results from Paper 3, CFL 40µg disc residue AI formulation